DEVELOPMENT AND VALIDATION OF A LIGHT WEIGHT, ENERGY DENSE, READY TO EAT (RTE) BAR

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Agriculture Specialization in Food Science

by

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November 2010

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
NOV 2010		**00-00-2010 to 00-00-2010**

4. TITLE AND SUBTITLE
Development and Validation of a Light Weight, Energy Dense, Ready to Eat (RTE) Bar

5a. CONTRACT NUMBER
5b. GRANT NUMBER
5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

5d. PROJECT NUMBER
5e. TASK NUMBER
5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
California Polytechnic State University,San Luis Obispo,CA,93047

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)
11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Providing additional calories in the form of an RTE bar to endurance athletes will increase performance and muscle re-synthesis, reduce muscle breakdown, and shorten recovery time. An RTE bar containing a blend of dairy proteins and carbohydrates will create a product with superior functionality, including bioactive and immunity enhancing properties from dairy derived ingredients. The protein will provide benefits in the form of easily digestible calories, essential amino acids and physical satiate. A formulation was developed and optimized, resulting in a final product that meets the required nutritional profile: 400kcal, 25grams protein per 100 gram serving size. The desired physical characteristics were achieved through processing by both conventional baking and freeze drying. The latter method improves the stability and functionality of the RTE bar. In order to meet the protein requirements of the RTE bar without compromising sensory properties, a unique protein source was developed. Using high concentrations of conventional protein sources like Whey Protein Concentrate (WPC) resulted in stale offflavors and unappealing textures. Milk Protein Precipitate (MPP) was developed for this formulation. MPP is a curd-like ingredient created through the combined heat and acid precipitation of dairy proteins. MPP can be used effectively in high concentrations provides a subtle dairy flavor. MPP delivers a balance of casein and whey, similar to that found in milk. The effectiveness of the RTE bar formulation as a post exercise recovery food was evaluated in a human studies experiment conducted on the Cal Poly campus. The human subjects study utilized 34 Cal Poly students in a single-blind cross-over design experiment. The study compared the effects of this high protein RTE bar against a calorically equal carbohydrate bar. The bars were administered after subjects completed the pre-assigned hikes on three consecutive days. Following the cross-over design subjects received the alternate bar in the second period of the experiment. Several blood markers involved in metabolism and inflammation were measured before and after the two treatment periods. No blood marker showed a statistically significant difference between bars, but several trends were observed. Body weight and fat percent were also unaffected by bar composition.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**137**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

COMMITTEE MEMBERSHIP

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Jacob Wilhelm-Maria Heick

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A formulation was developed and optimized, resulting in a final product that meets the required nutritional profile: 400kcal, 25grams protein per 100 gram serving size. The desired physical characteristics were achieved through processing by both conventional baking and freeze drying. The latter method improves the stability and functionality of the RTE bar.

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Keywords: milk protein, RTE, freeze drying, exercise recovery, lean muscle loss.

ACKNOWLEDGMENTS

I would like to acknowledge Dr. Hany Khalil for his role as a mentor during my undergraduate studies, and his instrumental contribution in bringing me into the Master's program. I would also like to acknowledge Dr. Rafael Jimenez for taking an active role during my research, stimulating my interest and exposing me to a multitude of engaging projects. I would like to thank Dr. Amy Lammert for her expertise in product and nutrition development, and for helping me to clarify my goals. Additionally I would like to acknowledge Dr. Steve Davis and the Kinesiology Department for help in the success of the Physiological Validation Study.

I would also like to thank the CSU Agricultural Research Initiative and Office of Naval Research (ONR) for the funding that made this research possible. I am grateful that I was given the opportunity to continue my studies and hope that I have made a positive contribution. I would also like to thank California Dairy Inc (CDI) for their commitment to furthering the dairy industry, and their contribution to my research.

"There are two ways to slide easily through life: to believe everything or to doubt everything. Both ways save us from thinking." Alfred Korzybski

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LIST OF ACRONYMS AND TERMS

BCAA – Branched Chain Amino Acids

BMP – Butter Milk Powder

Cortisol AM - Hydrocortisone

CPK – Creatine Phosphokinase

CRP – C-Reactive Protein

EAA – Essential Amino Acids

EPO – Erythropoietin

EVOH - Ethylene Vinyl Alcohol

FFM – Fat Free Mass

GRAS – General Recognized As Safe

HFCS – High Fructose Corn Syrup

MFGM - Milk Fat Globule Membrane

MRE – Meal Ready To Eat

MPP – Milk Protein Precipitate

PDCAAS - Protein Digestibility Corrected Amino Acid Score

PE - Polyethylene

PER - Protein Efficiency Ratio

WPC – Whey Protein Concentrate

RTE – Ready To Eat

RTEM – Ready To Eat meal

RDA – Recommended Dietary Allowance

SMP – Skim Milk Powder

1. INTRODUCTION

The objective of this project was to develop a novel ready-to-eat (RTE) bar to positively influence exercise recovery through muscle re-growth. Detrimental health effects of over-exertion during physical activity include loss of lean muscle mass and inflammation. These symptoms are found among both combat military personnel and endurance athletes. This research aims to address these negative health impacts of over-exertion through the development of a customized RTE bar, positively impact functional nutrition by tilting the daily energy balance and thereby reversing the negative effects of a caloric imbalance. In addition, the RTE bar was formulated to contain 50% of a Recommended Dietary Allowance (RDA) serving of complete protein.

The nutritional profile of the RTE bar is modeled on a small meal or snack which will provide a balanced blend of macro and micro nutrients. The 100 gram bar provides 400kcal and 25grams of protein in one serving. In order to meet these goals different protein sources were researched with a focus on the nutritional benefit of milk and dairy-derived ingredients. In order to provide protein in a RTE bar at the stated high concentrations without compromising its sensory quality, a novel protein source was developed. This protein ingredient was shown to be an effective method of delivering the needed macronutrients.

In order to validate the RTE formulation a human subjects study was conducted on the Cal Poly campus. The study utilized the high protein RTE bar and a control carbohydrate bar. The experiment was designed to mimic combat soldier activity in mountainous terrain. Several different response variables were taken during and after the

physical activity. The goal was to measure the influence of the exercise on concentration, inflammation, body composition, and peak power. Statistical analysis revealed no significant difference between the control carbohydrate and high protein RTE within the blood markers analyzed. However, the blood markers indicated that the exercises did induce inflammation in the subjects.

2. LITERATURE REVIEW

Health Concerns

Nutritional Concerns in the Military

The military has long been concerned with the health and performance of its soldiers. This is witnessed by the special programs and institutions the military has dedicated to medical research. Examples of these include the Office of Naval Research, Army Research Institute of Environmental Medicine, US Army Natick Soldier Center, and the Institute of Medicine U.S. Army Medical Research and Nutrition Laboratory. The military's main nutritional concern is sustaining and enhancing the physical and mental performance of soldier's through diet (Anonymous 1994). During combat or regular training soldiers expend from (3109 kcal to 7131kcal) per day while consuming on average only 3000kcals per day (Tharion et al. 2005). There are many reasons for these extreme dietary deficiencies including: loss of appetite, lack of time and portability issues. If not properly addressed, this energy imbalance can lead to loss in lean muscle mass, and impaired physical and cognitive performance (Marriott 1995).

A loss of fat free mass (FFM) can also be interpreted as a loss in lean muscle. This was demonstrated on Italian soldiers in a body composition and physical exercise study (Malavolti et al. 2008). It was reported that the soldiers lost an average of 4.02kg +/- 1.42kg in FFM during the first three months of the experiment. This portion of the experiment contained increasingly strenuous exercises in the gym and in combat simulations, and was designed to represent ground combat with uncontrolled diets. The results of this experiment reflect the kind of situations that affect active duty soldiers

subjected to strenuous exercise. The effect of a negative energy balance has been investigated by numerous studies over the years. Observations include, but are not limited to: large losses in body mass, physical and mental fatigue, muscle soreness, weakness during recovery, impaired group function and loss of motivation (Montain and Young 2003). While the extent of the physical or mental impairment fluctuated between the different tests, the general consensus is that the performance of the soldiers was negatively affected. Each research group applied its own levels of nutrients (fasting through 3600kcal per day) for varying periods (5 days - 6 months) as well as using different tests to register the response (time to complete run versus hand grip) (Montain and Young 2003).

To avoid the effects of a negative energy balance, adequate calories must be consumed. To provide this nutrition in a way that is practical, as weight and space are constraints, small energy dense meals are seen as a solution. This being the case, fat would seem to be the ideal supplement for a military ration. Fat provides 9kcal/gram versus 4kcal/gram for carbohydrates or protein (Montain and Young 2003). However, supplementing the diet with additional calories from fat does not lead to significantly greater performance or even increased lipid metabolism (Hoyt et al. 1991). A study by Hoyt et al. 1991 indicated that while fat contained in the supplement provides additional calories, it is not readily metabolized and does not reverse the effects of underfeeding. The Committee on Optimization of Nutrient Composition of Military Rations for Short-Term, High-Stress Situations 2006 recommended a protein level of 1.2-1.5g per kg of body weight or 100-120g of protein per day. This is needed in order to maintain adequate serum levels while reducing net protein loss through sparing muscle protein breakdown.

This would be a large amount of protein to deliver in a single serving. So it was decided that a small energy dense ration, which could provide 25-30% of this amount would be ideal.

Sarcopenia

Sarcopenia is a muscle dilapidation disease that affects up to forty five percent of those over the age of sixty five (Cribb 2006). While the mode of action of the disease is not well understood, the effects are being increasingly investigated. Sarcopenia is diagnosed as a loss of lean muscle mass with a corresponding increase in body fat (Evans 2010). While sarcopenia refers directly to the loss of muscle in the elderly, treatment and research also investigate the young to better identify the causes of lean muscle loss. Lean muscle is the bulk tissue of the body that is responsible for movement and represents an energy source other than body fat or glycogen. Muscle is composed of protein and thus represents the body's storage of amino acids that are utilized not only in metabolism but also numerous other physiological processes. Loss of skeletal muscle results from an imbalance between muscle protein synthesis and degradation (Evans 2010). The cause of imbalance will vary for the specific demographic. For young persons it could be the result of over-exertion without adequate calories, while for older individuals it could be from a reduction in physical activity accompanying declining health and a poor diet. The mode of action for sarcopenia is the loss of the ability to convert available amino acids to glutamine, causing the body to increase anabolism of the liver to meet the demand of glutamine (Cribb 2006). For individuals consuming a hypocaloric diet, higher levels of dietary protein are required to reduce these detrimental effects (Lemon 1987).

Muscle protein is the most important body protein for endurance athletes (Tipton and Wolfe 2004). As the working site of movement, the muscle represents a major consumer of energy and the largest site of lipid oxidation and glucose metabolism (Cribb 2006). In order to reverse damage or stimulate muscle anabolism, net protein synthesis must exceed protein breakdown. In order to achieve this, a balance of macronutrient intake and resistance exercise must be introduced into the lifestyle (Cribb 2006). These two factors work synergistically providing a net gain in lean muscle mass that is greater than if each factor worked independently. During resistance exercise, the consumption of protein-rich dietary meals can be a major factor in maintaining or increaseing muscle mass (Phillips et al. 1998).

Cachexia

Cachexia is a complex metabolic condition that is associated with concurrent chronic diseases such as AIDS. Cachexia may affect any age group and is characterized by muscle wasting with or without body fat loss. Cachexia appears to selectively target actomyosin and thus heavily targets skeletal muscle (Evans et al. 2008). It appears cachexia can be reverted by therapies which reduce muscle inflammation and directly influence skeletal muscle growth in patients (Evans 2010).

Gastrointestinal Health

Diarrhea and other gastrointestinal problems have been associated with both military personnel and endurance athletes. The causes of these problems have been linked to stress, nutrition, and the physiological effect of exercise on the digestive system. One hypothesis is that during periods of extreme exertion blood flow is directed toward the active muscles, thus temporarily dehydrating the gut and increasing its sensitivity to

stress (Ha and Zemel 2003). Another theory relates the problems to fructose consumption. The Committee on Optimization of Nutrient Composition of Military Rations for Short-Term 2006 recommends limiting the amount of fructose in rations to below 25g. Fructose at higher levels than this may contribute to gastrointestinal problems (Anonymous 2006). High fructose corn syrup (HFCS) is a common ingredient in food formulations, particularly in bar and supplement products frequently used by athletes. The large quantities of fructose consumed directly from these products might contribute to the GI problems seen in these individuals.

Dairy products have historically been associated with gut health and research has identified whey protein as one contributing factor. Whey proteins provide glycomacropeptides that are potentially utilized as prebiotics, which stimulate the growth of probiotics. Glycomacropeptides may also activate cholecystokinin which has many physiological effects such as the regulation of food intake and the release of pancreatic enzymes (Dockray 2009). Milk also contains prebiotics and is commonly associated with Lactic acid bacteria, the major family of probiotics. Probiotics are associated with promoting gastrointestinal and immune system health as well as the synthesis of vitamins (Hazen 2009). The benefits of probiotics result when viable organisms reach the small intestine in sufficient quantities thereby positively influencing the microflora of the small intestine. For this to occur, the organisms must be able to survive the initial processing of the food product and its eventual digestion in the mouth and stomach (Fernández et al. 2003).

Protein And Sport Nutrition

Protein Requirements for Athletes

All biological proteins are assembled from twenty amino acids. They can be combined in numerous sequences to form the complex and diverse array of proteins seen in living systems. The defining characteristic of a protein is its vital amino nitrogen group. In addition, proteins are the only macronutrient to contain nitrogen (Anonymous 2005). Proteins and amino acids are vitally important components of the body because they function as cell membranes, hormones, enzymes, vitamin precursors and nucleic acids. With its diverse functions and interdependence, dietary protein is essential for health, reproduction, growth, and maintaining of homeostasis. Protein is a necessary component of the human diet. Currently the recommended daily allowance (RDA) is set at 0.8 grams protein/kg of body weight for the healthy average adult or 50grams protein per day (Anonymous 2005).

There is a long-standing theory held by many athletes, coaches, supplement companies, and nutritionists that athletes need additional dietary protein. The logic being that proteins and amino acids are responsible for the synthesis and replacement of the structures associated with exercise and muscle building (Nemet and Eliakim 2007). Those who are more active would need more protein for fuel and rebuilding. Logic notwithstanding, there is little scientific proof that athletes require additional protein and some studies have even demonstrated that athletes require less protein (Phillips et al. 2007), however the assumption remains. The U.S and Canadian agencies responsible for the RDA have considered an increased consumption of protein of 1.2-1.4 grams protein/kg of body weight to be beneficial to endurance athletes (Tipton and Wolfe

1998). However, they have not stated that athletes actually require this increase. Interestingly enough, most athletes already consume an excessive amount of protein, more than the RDA and even more than the increased RDA. Diet surveys on strength and power training athletes have estimated consumption levels as high as 2-3 grams protein/kg of body weight with endurance athletes consuming approximately 1.2-1.5 grams protein/kg of body weight (Phillips et al. 2007).

Protein Balance

Energy balance is an important concept for individuals trying to modify body weight or composition. This refers to the difference between calories from food consumed (input) and the calories expended by physical activity (output). Tilting the balance either way will alter one's lean muscle mass; consuming more calories than one expends leads to a net gain in weight and consuming less leads to a net loss (Benardot and Thompson 1999). A study by Robert Demling and Leslie DeSanti in 2000 worked with overweight police officers and found that the subjects' average daily protein intake was below the RDA. This low intake was likely a factor in the lean mass and strength loss experienced by the individuals. In order to increase muscle mass or reverse lean muscle loss, the nutritional goal would be to tip the nitrogen balance to the positive side by consuming a net positive intake of amino acids (Phillips et al. 2007).

One of the most important indicators of protein utilization in the body is the Nitrogen Balance, which is defined as the minimal amount of protein ingested that will balance all nitrogen lost (Tipton and Wolfe 2004). The Nitrogen Balance is what was used to calculate the RDA for protein and amino acids. This method, however, is tailored to find the minimum intake level necessary to limit deficiency and not for optimal

athletic performance (Phillips et al. 2007). Additional protein can be rationalized because all ATP expended for bodily movement must come from energy stores (Lemon 1987). Muscle and skeletal protein represents a small "pool" of reserve energy that can be utilized during physical activity in addition to glycogen and lipid stores. Because this pool cannot be expanded, there is no other way to store the amino acids (Phillips et al. 2007).

There are restraints on the quantity of protein that can be consumed causing any excess protein to simply be stored as fat (Nemet and Eliakim 2007). Consuming surplus protein can also be a problem because of the nitrogen that is inherent in its structure. Nitrogen can be toxic and in excess will be converted into urea (Phillips et al. 2007). On the other hand, the body also reacts to high protein levels by increasing amino acid catabolism. During exercise the body's metabolism switches to a predominantly catabolic state. After exercise, during rest, the body shifts more towards anabolism (Tipton and Wolfe 1998).

Amino acids in muscle building

High quality proteins like eggs, dairy products and muscle proteins contain all of the twenty amino acids. This has been demonstrated with research showing that whole or skim milk consumption leads to a greater positive muscle protein balance and net amino acid uptake than soy based milks (Hartman et al. 2007). Amino acids are also the precursors to physiological compounds like creatine, epinephrine, and purine bases (Nemet and Eliakim 2007). Amino acids can provide ATP for muscle contraction through direct oxidation or the conversion to glucose via gluconeogenic pathways. In addition, the availability of the necessary amino acids is a requirement for muscle protein

synthesis (Levenhagen et al. 2002). Blood amino acid concentration has physiological signaling qualities like growth hormone, insulin, and insulin-like growth factor. This is dependent on the quality of the protein and the specific amino acids consumed (Nemet and Eliakim 2007). These amino acids function as regulatory molecules to stimulate muscle protein anabolism.

Decreasing blood amino acid concentrations has been shown to result in decreased muscle protein synthesis, while increasing the concentration has restored the synthesis rate (Tipton and Wolfe 1998). The physiological response changes depending on the protein type, differing even between two high quality proteins like whey and casein. Blood amino acid concentration is higher and adjusts more quickly after consuming whey protein, but anabolic response is greater with casein (Tipton and Wolfe 2004). There is a notable difference in the resulting blood amino acid concentration after ingesting intact proteins when compared to hydrolyzed amino acids (van Loon et al. 2000).

Muscle catabolism is an integral part of growth. As the muscle contracts, muscle fiber damage occurs. The muscle is the site where the metabolism responsible for this movement occurs, and as a result, the increase in amino acid oxidation likely occurs in these sites as well. During rest, muscle anabolism occurs and the previously damaged muscle is rebuilt. Muscle contraction leads to skeletal, structural, and membrane protein damage, proportional to the extent of the physical activity. The eventual muscle anabolism leads to a greater need for available amino acids for the synthesis of new proteins (Levenhagen et al. 2002). Supplementation of energy in the form of carbohydrates and/or fat can provide the energy necessary for the exercise and post-

exercise glycogen synthesis. Amino acids are, however, necessary for muscle protein re-synthesis. Muscle synthesis is influenced by the intramuscular availability of amino acids as well as blood flow. An increase in muscle synthesis increases the transport and delivery of amino acids to the muscles. The availability of these amino acids, either from the diet or resulting from muscle breakdown, may act as a signal for the eventual muscle synthesis (Tipton and Wolfe 2004).

Essential Amino Acids

Essential amino acids (EAA) are those that cannot be produced in sufficient amounts by the body, but are found in high quality protein sources (Nemet and Eliakim 2007). There are nine EAAs: Lysine, threonine, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan and histidine. Two of these (lysine and threonine) cannot be synthesized by the body and therefore must be consumed in the diet (Bos et al. 2000). EAAs are even more critical to the synthesis of muscle protein and represent a limiting factor in protein synthesis (Cribb 2006). Animal studies have shown that muscle synthesis is reduced when EAAs are withdrawn from the diet. The EAA content of a protein is seen as the indicator of the quality of the protein source (Table 2-1), EAAs, which include branched chain amino acids, stimulate lean muscle protein synthesis (Ha and Zemel 2003).

Table 2-1: EAA's in selected protein sources adopted g/kg air-dry wt (Rutherfurd and Moughan 1998)

EAA ***BCAA***	Soy Protein Concentrate	Soy Protein Isolate	Whey Protein Concentrate	Milk Protein Isolate
Threonine	26.1	34.1	57.9	40.2
Valine	***33.9***	***44.4***	***49.1***	***61.1***
Methionine	10.0	12.6	21.8	29.1
Isoleucine	***31.5***	***43.1***	***52.2***	***49.5***
Leucine	***54.2***	***71.0***	***88.2***	***94.4***
Phenylalanine	36.0	48.1	29.5	48.4
Histidine	19.3	26.0	17.2	31.8
Lysine	42.6	60.3	72.8	75.9

Branch Chain Amino Acids

There are three branched chain amino acids (BCAA): leucine, isoleucine, and valine. They are a unique subset of the essential amino acids, accounting for 35% of the EAAs in muscles (Shimomura et al. 2004). BCAAs differ from other amino acids in that they are directly utilized in skeletal muscle as a source of energy (Nemet and Eliakim 2007), and show significant oxidation during exercise. This unique ability may increase the availability of carbohydrates and reduce the impact of muscle breakdown during exercise (Walzem et al. 2002). Endurance exercise shows an increase in the amino acids' oxidation, supporting the theory that BCAAs are of particular importance to endurance athletes (Phillips et al. 2007). BCAAs can also contribute to glucose production through the Cori cycle, due to their ability to form transaminase pyruvate in the muscle as an intermediate to alanine (Nemet and Eliakim 2007). BCAAs have also been shown to reduce exercise-induced muscle damage and increase the synthesis rate (Shimomura et al. 2004).

Carbohydrates and Exercise Metabolism

Exercise Recovery

Some studies have shown that combining protein with carbohydrates in post-exercise meals can improve recovery time (Zawadzki et al. 1992; Levenhagen et al. 2002). Others report that the combination has no positive synergistic effect when compared to just carbohydrates alone (Jentjens et al. 2001). Much of the disagreement on the effects of combining protein with carbohydrates for improved recovery is due to the quantity of protein or carbohydrates provided and the style and extent of the exercise, as well as the method of measurement. In a study that resulted in a zero net gain in muscle glycogen synthesis, blood insulin levels increased when protein and carbohydrates were administered (Jentjens et al. 2001). Yet other studies found a net gain in muscle synthesis if protein was included in the supplements (Zawadzki et al. 1992; Kimball et al. 2002). This could indicate that insulin level may not be the rate-limiting factor in muscle glycogen synthesis but are affected by protein consumption (Jentjens et al. 2001). In this case muscle synthesis will be achieved as long as adequate carbohydrates are provided. Even without additional protein intake, nitrogen balance may be restored with only the consumption of carbohydrates (Phillips et al. 2007). A protein-sparing effect occurs if sufficient carbohydrates are available, and protein oxidation will be ignored or reduced. In low carbohydrate diets, protein would be redirected for utilization as fuel instead of its anabolic use (Benardot and Thompson 1999). As stated before, the major energy sources during exercise are lipids and carbohydrates (glycogen), while protein or amino acids account for only 3-6% of the ATP needed during exercise.

Endurance athletes generally “carbo load” consuming large quantities of carbohydrates before periods of extreme exercise. Consuming 7-10 grams of carbohydrates per kg of body weight is recommended for those participating in marathon events (Nisevish 2008).This “loading” leads to larger concentrations of available carbohydrates which are stored as glycogen, and if protein is also consumed, it improves the net protein balance and reduces protein utilization (Gibala 2007). It is becoming understood that amino acids play an important role in the intermediate steps of the TCA cycle (Gibala 2006). There is the potential for athletes to reduce stored fat and alter their body composition through consuming a low calorie diet, skewed towards higher protein consumption (Phillips et al. 2007). Carbohydrates, if consumed in excess without adequate activity, are particularly prone to be stored as fat, and developing excess fat can lead to additional health problems (Demling and DeSanti 2000).

Glycogen

Glycogen is the body’s natural energy storage form for carbohydrates. It is the first and major energy source utilized during physical activity; however it is finite in quantity and must frequently be replenished. Glycogen represents a relatively small store of energy, approximately 1500-2500kcal when saturated; this is due to the low energy density of carbohydrates (Hoyt et al. 1991). After exercise, to restore glycogen levels to pre-exercise levels, an estimated supplement containing between 1-1.2 grams carbohydrate per kg of body weight is required (Phillips et al. 2007). Most evidence suggest that if adequate carbohydrates are consumed (>1.2 gram/kg body/ hour) the benefits of additional protein are negated. However, when protein is ingested with

carbohydrates, glycogen synthesis rate will increase if the quantity of available carbohydrates is low (Gibala 2007).

Effect of Timing

Consensus among athletes, trainers, and nutritionists is that consuming supplemental protein and carbohydrate at the end of exercise provides a better anabolic environment (Nemet and Eliakim 2007). One study found supplementing within one hour of exercising promoted greater gains in lean muscle mass compared to either soy or carbohydrate controls (Hartman et al. 2007). Immediate post-exercise supplementation could benefit the endurance athlete in repair and synthesis of muscle protein and the reloading of glycogen (Gibala 2007). Protein consumption, whether consumed alone or in conjunction with carbohydrates, will be a major determinant in strength or muscle mass gains (Phillips et al. 2007).

Protein Supplementation Case Studies

Under conditions of weight loss, diets that contain more protein have been shown to lead to significantly less lean-muscle loss compared to diets high in carbohydrates (Layman et al. 2005). One study on protein utilization found that subjects who were deficient in initial glycogen stores before endurance exercise utilized more protein as a percentage of total energy expended (Lemon and Mullin 1980). In a study comparing high and low protein diets with two exercise treatments, those who consumed the high protein diet lost more body fat without disrupting HDL cholesterol levels (Layman et al. 2005). This seems to indicate that increasing protein in the diet could potentially improve the body composition of subjects during exercise. Another study was conducted comparing a control containing only carbohydrates and fat to a treatment that also

incorporated protein. The results showed a twenty percent greater quantity of circulating amino acids (lysine, valine et al.) in the blood after exercise with the protein supplement (Levenhagen et al. 2002). The presence of the protein in the supplement seemed to reverse the catabolism that was seen with the control supplements. The hypotheses is that the limiting factor in muscle protein synthesis is not the overall energy consumed in the diet, but the amino acid concentration in the body as a result of the food consumed.

Dairy as Functional Nutrition

Milk Overview

Milk provides all the nutrients necessary for the growth and development of the maturing mammal. Milks supply macronutrients as well as immunity compounds and micronutrients (Walzem et al. 2002). The composition of milk varies depending on the species, stage of lactation, season, and a variety of other factors. Milk contains a combination of two major protein groups, wheys and caseins, each has specific functional and nutraceutical properties.

Bovine milk contains on average 3.4% protein, which is primarily 80% casein and 20% whey (Spreer 1998). Casein and whey proteins behave differently during processing and digestion. Casein will coagulate in the stomach forming clots that are harder for enzymes to proteolysie; however once in the small intestine they are absorbed quite readily. Whey proteins do not coagulate on contact with the stomach's acid and are thus transferred quickly to the small intestine where they slowly become absorbed over a much greater length of time (Walzem et al. 2002).

Milk has been shown to be an effective functional ingredient for promoting positive health and athletic performance. A study comparing a carbohydrate and soy supplement to a skim milk one in a controlled laboratory weightlifting experiment showed that skim milk increased the type I and type II muscle fiber areas greater than the soy and carbohydrate products. Skim milk also increased the fat and bone free mass above that of the other treatments, and led to a greater reduction in fat mass (Hartman et al. 2007). Milk's protein profile is unique in containing all essential amino acids and high concentrations of BCAAs. Casein and whey have separate profiles, but even independently they score high compared to other protein sources (Table 2-2).

Table 2-2 - BCAA composition of selected proteins, adopted form (van Loon et al. 2000).

% by Wt	Casein	Whey	Pea	Wheat
L-Isoleucine	5.8	5.1	2.4	2.6
L-Leucine	10.1	8.7	5.1	5.6
L-Valine	7.4	4.5	2.7	3.0

Casein

Casein accounts for 80% of the protein in bovine milk. It is the fraction that is responsible for creating cheese because it is hydrolyzed by chymosin and its solubility is influenced by pH. Casein proteins have been shown to contain various peptides that have bioactive properties, and these peptides seem to require proteolysis of the main casein forms in order to be released (Shag 2000); (Walzem et al. 2002). In the study of overweight police officers by Robert Demling and Leslie DeSanti in 2000, after twelve weeks, lean muscle gains were doubled and fat loss was fifty percent greater in the group which was fed a casein supplement compared to the whey group. Casein has four major subgroups (α_{s1}, α_{s2}, β, κ), each has multiple bioactive peptides with different abilities and

strengths. There appear to be several main substrates that are affected by these peptides (Shag 2000). Opioids, known as casomorphins, have properties similar to that of opiates and have been seen to increase gastrointestinal transit time among other physiological effects. Immunomodulating peptides have been shown to affect T-cells and macrophage activity. In addition, antihypertensive, anticariogenic, and antithrombotic properties have been observed. Hydrolysate components of casein have been shown to decrease amino acid oxidation and net protein breakdown, leading to improved nitrogen retention compared to other supplements available commercially (Demling and DeSanti 2000). Unlike whole or native protein, hydrolysates have also been shown not to stimulate the release of the hormone cortisol, which has lipogenic and catabolic properties.

Whey

Whey proteins represent the minor portion of total milk protein, accounting for approximately 20% of the total. Whey protein exists at the same concentration in human milk as in cow milk. However, human milk contains no β-lactoglobulin and cow milk has a much lower level of lactoferrin than human milk (Bos et al. 2000). Whey protein has also been identified as a possible source for bioactive peptides. After ingestion, whey protein leads to a very rapid oxidation and whole body protein synthesis. Casein, on the other hand, leads to whole body proteolysis suppression (Hartman et al. 2007). Whey is composed of several protein fractions including β-lactoglobulin, α-lactalbumin, proteose-peptones, and blood proteins (Walzem et al. 2002). The majority of these peptides seem to have influence on the immune and digestive systems such as chelating, antimicrobial and antioxidant activity. In addition immunoglobulins have potential anticancer and antitumor effects (Shag 2000). Whey is

also said to have hypocholesterolemic properties which might actually "balance" out the possible negative health effects of the saturated fat naturally occurring in milk (Walzem et al. 2002). Whey protein contains a high proportion of sulfur-containing amino acids (cycteine, methionine), which are said to contribute to the higher protein efficiency ratio (PER) of whey. Whey may also lead to the sparing of tissue proteins ordinarily used in response to immune challenges (Walzem et al. 2002). Whey proteins contain high amounts of EAA and BCAA which are generally lacking in plant and other protein sources (Table 2-1). As a byproduct of cheese production the whey stream is seen as a rich source of BCAA, equaling at least 26% of the total amino acids present (Bos et al. 2000). The amino acid composition of whey is said to be relatively similar to that of skeletal muscle, making whey a good source of amino acids during muscle re-synthesis.

Minor Components

Components beyond the macronutrients of milk, such as minerals and carbohydrates, have also gained recent attention. Lactose has the ability to form oligosaccharides which have both specific and broad prebiotic properties. These oligosaccharides can be labeled Generally Recognized As Safe (GRAS) for use in products desiring enhanced probiotic effects. Lactose may also influence the absorption of calcium, which in turn is said to have a role in regulating blood pressure. Milk enzymes do not appear in finished products as they are deactivated during pasteurization. However there is emerging research on particular enzymes like lactoperoxidase which is used as a preservative in some products (Walzem et al. 2002). Lactoferrin, another milk enzyme of interest, has iron-chelating, cation transport, and anti-infectious properties. Lactoferricin, a form of lactoferrin, also has bactericidal activities (Bos et al. 2000).

Buttermilk, the by-product and liquid phase of butter manufacturing, has been seen as a source of potential bioactive components. Sphingomyelin and phospholipid have been demonstrated to have anticancer properties and are concentrated in the buttermilk fraction (Walzem et al. 2002).

Nutritional Bar Development

Target Formulation Constraints

The goal of developing this RTE bar is to supply high energy and a designated percentage of the RDA of calories, as well as a combination of all of the macronutrients. Incorporating dairy protein into a RTE bar is a preferred method of directly reversing the negative effects of lean muscle loss through the diet. Development of the RTE bar focused on delivering the maximum nutritional functionality to the end-user.

The form and source of protein in a food product is of great importance, in that it must appeal to the target market, meet nutritional objectives and function appropriately in the formula (Hazen 2008). The quality of protein consumed is very important for maximizing the anabolism of muscle protein. The high-quality proteins in milk, dairy products, eggs, and muscle meats are ideal (Phillips et al. 2007). Another measure of protein quality, without measuring the concentration of individual amino acids is the Protein Digestibility Corrected Amino Acid Score (PDCAAS) (Hazen 2008) (Table 2-3). From this perspective, dairy protein, in particular whey protein, appears to be best suited for a protein bar product. In addition to their nutritional properties, whey proteins have critical functional properties that make them practical in bar formulations. They retain moisture, have a mild flavor, contribute to extended shelf life, lead to reduced

cooking/baking losses, and can be used to replace carbohydrates (Runestad 2004). The quantity of high-quality protein is also important; 20-25g appears to be the upper limit to stimulate muscle protein synthesis, and would be an ideal maximum in a single-serving product. Above this level amino acid oxidation and urea formation become more prevalent (Phillips et al. 2007), diminishing the effectiveness of adding protein. Currently, the military serves Meal Ready to Eat (MRE) to soldiers in the field. These are lightweight and contain several separate packages that represent a full meal when eaten together. One concern with the use of MRE's is that the macronutrients are not evenly distributed in the different components. This allows the soldiers to "field strip" or selectively eat portions of the ration and therefore not gain all the intended nutrients from the meal (Anonymous 2006). The goal of an optimal nutrition bar is to be a high energy snack or small meal that provides a designated percentage of the calories and all macronutrients needed by an individual in a day.

Table 2-3: Protein Comparisons by Source: PDCAAS (Hazen 2008), Biological values (Runestad 2004)

Source	PDCAAS Value	Biological Value
Whey	1.0	104
Egg	1.0	100
Soy	1.0	74
Pea	0.86	-
Hemp	0.46	-
Wheat	-	54

The Committee on Optimization of Nutrient Composition of Military Rations for Short-Term, High-Stress Situations 2006 lists the following recommendations for the development of a ration:

- Protein and carbohydrates are the priority
- Fat is important for palatability and absorption of fat soluble vitamins
- Weight and volume restriction of: 0.12 cubic feet, 1.36kg
- Shelf life of 2-3 years
- Individual portions that can be easily distributed in backpacks
- Palatability is a primary concern
- Variety of familiar flavors, colors and textures
- Potential for either sweet or savory formulations.

Nutrition bars are among the easiest products to fortify. They have an easy dry mixing stage, low thermal processing (if any), and they generally utilize opaque laminate packaging (Hazen 2009). The main challenge with the formulation of nutrition bars is the drying and hardening that occurs during storage and throughout the shelf life (Runestad 2004). This problem is compounded by the long shelf life and humidity standards set by the military, as well as the moisture and water activity in the bar. Water activity (aw) is an important property of foods that will help dictate food safety, shelf life and textural parameters. The water in the product migrates over time to the protein and the dry ingredients, which will alter the intended texture (Hazen 2010). Higher aw will result in a softer bar, however there is a limit to this as shelf-stable bars need to be at an aw level of less than 0.65 for food safety reasons (Hazen 2010). A consideration that should be taken into account with an RTE bar is that protein metabolism requires more

water than either lipids or carbohydrates (Lemon 1987). This could be problematic for a product that contains high protein levels but low moisture content. This makes the option of hydrating the RTE bar a more effective means of delivering the desired product nutrients.

Ingredients

Dietary Fiber

Dietary fiber is an interesting ingredient from a formulation perspective. It is desired because it has minimal nutritive properties. Dietary fiber under the current definition pertains to fibers that are indigestible but can be utilized as a prebiotic fiber in the small intestine. Fiber can also be an important component in a bar formulation, providing necessary nutrition and digestive functionality. Fibersol-2 (see appendix page 131) is a commercial ingredient which is labeled as a resistant starch, it provides dietary fiber and helps with texture throughout the product shelf life (Runestad 2004).

Delactosed Permeate

Delactosed permeate (see appendix page 130) is a novel dairy ingredient developed using the waste stream of WPC concentration. It has a high mineral concentration ~ 30% ash, with high calcium content at 3.7%. Delactosed permeate contains oligosaccardies and many micronutrient ingredients. Therefore it could be used to boost calcium and vitamin content in a particular formulation targeted to women or the elderly. There is also recent research that points to success in using Delactosed permeate as a salt replacement in bakery products. This would help the product become more

attractive to individuals struggling with hypertension or individuals simply looking for low sodium foods.

Flavor

From the onset of this project there has been the concept of developing a savory form of the RTE bar. The majority of bars that are on the market today are sweet (Hazen 2009). While there is interest in the concept of a savory bar, there is little indication that the market would accept it. This is likely a result of the current standard formulations used by most producers and expected by consumers. Currently available bars contain significant amounts of HFCS to act as a binder, or contain carbohydrates as a major ingredient, and often use bitter tasting protein blends. These qualities lend themselves more to a sweet formulation than a savory one. However, considering the specification of the RTE bar as high protein with a mild dairy flavor, a savory option might be achievable.

Bar Processing

Freeze Drying

Freeze drying preserves food by removing free and bound water. It has many commercial and industrial applications and is used in the processing of high value and biologically active products (Oetjen and Hasely 2004). Freeze drying may be the processing method of choice for the RTE bar because of its low processing temperature. Freeze dried products are easy to rehydrate and still retain biological activity. Freeze drying relies on the properties of water sublimation, that is the bypassing of the liquid phase in the transition from a solid state to a gaseous state, to remove the water from the

product with minimal heat input. Sublimation occurs when the partial pressure of the environment is below that of the product so the frozen water must evaporate to create equilibrium. However, the water vapor is constantly being removed by the condensation coil which maintains sublimation. A basic freeze dryer contains four parts (Jennings 1999):

- A chamber that can be both temperature and pressure controlled
- Vacuum pump which lowers the pressure and removes some gases
- Heating plates that provide heat to increase the sublimation rate
- And a refrigerated coil that removes the sublimated vapor from the chamber's environment by creating a temperature gradient.

To freeze dry, first the product must be completely frozen to a very low temperature. This is generally done in the blast freezer at a setting of -14^{o}F. The sublimation of the frozen water occurs after the samples are placed in the chamber and the heating plates and vacuum are set to the desired levels. Without being placed in the pressure-controlled atmosphere, the ice would simply melt. The heating plates provide enough minimal radiant heat to supply the latent heat of sublimation (1075 BTU/LB or 2495.08 KJ/KG). Secondary drying occurs after all free water has been removed; the product will appear dry but still contains bound water. This water can also be removed with resulting theoretical moisture content between 1-5%.

3. BAR FORMULATION AND MANUFACTURING

Introduction and Experimental Logic

Stable energy balance and physical health are critical factors for certain high risk groups like soldiers and endurance athletes. Such individuals depend on their strength and stamina to do their jobs and often have restricted carrying capacity, limited time to eat, and unbalanced meals. Stress has a profound effect on the human body which is compounded by a poor diet. If appropriate safeguards are not taken there is the potential for lean muscle loss and long-lasting physical and psychological deterioration. A reduced calorie diet, especially one that lacks high quality protein, is one of the main causes of this physiological stress. There is the possibility of mitigating this risk with a well-balanced high protein (RTE) nutrient bar. The specifications (Table 3-1) for such a product are designed to provide the required high energy nutrients within the time and space constraints.

Table 3-1: RTE bar physical and nutrition specifications

Ready To Eat (RTE) Bar Specifications	
Weight: 100g	Intermediate moisture range
Total Calories: 400kcal per serving	Nutrient and product stability
Protein Content: 25grams protein	Contains a majority of dairy ingredients

To meet the above specifications, the ingredients must be carefully selected, particularly the protein source. Based on the current scientific understanding of protein metabolism, providing a high quality protein (Table 2-3) would be the most effective method for achieving this. This would ensure that any and all amino acids would be

available in adequate amounts to support the anabolism of muscle. Dairy protein was considered the best option, for the RTE development and more specifically, whey protein due to its extensive use in similar commercial products, and the relatively high concentration of BCAA (Table 2-2) involved in muscle contraction. However, whey protein is just a fraction of milk, lacking casein and dairy lipids. Considering this we undertook to develop a more suitable protein source from milk to provide the needed protein profile, which could offer superior nutritional benefits to whey protein alone.

In addition to the protein type, the specific processing method for such a bar is important. The military has stringent guidelines for their current rations which include a 2-3 year shelf life, nutrient stability, and small compact size (Anonymous 1994). In order to meet these specifications, freeze drying was chosen as the processing method for the RTE bar. More common drying processes, such as baking, vacuum, and air drying, were also investigated. Several micronutrients and probiotics were then considered as possible additives to the basic RTE formulation. Probiotics are of growing interest to the food and dairy industries (Stanton 2001), consumers are becoming aware of probiotics and demanding them in foods they commonly eat. Processors are also recognizing the potential of probiotics to increase their market share and provide a novel method of delivering targeted nutraceutical properties (Ouwehand 1998). Probiotics would be an ideal addition to a nutritional bar formulation; the challenge is ensuring the survival of the active probiotic organisms which are limited by the relative instability of organisms during processing and storage.

Materials and Methods

List of Ingredients

- Hilmar 8200 whey protein concentrate (WPC): protein source and filler. Typical: 82.5%, Specification: 80.0% min Protein.
- Milk Protein Precipitate (MPP): Ricotta-like cheese manufactured in-house, protein source, binder and filler. Protein 22-27%, Moisture 35-40%.
- Buttermilk Powder (BMP) Dairy America: protein and micronutrient source. Protein 32 - 34.5%, Fat 5.5 - 6.0%, Lactose 49.0 – 50.5%.
- Non Fat Dry Milk (SMP): filler and control ingredient to BMP. Protein 36%, 50% lactose.
- Bread flour (high gluten): filler and carbohydrate source. 73% carbohydrate, 12% protein.
- Sucrose: Filler and sweetener.
- Non-Iodized Salt: Flavor enhancer, water activity control.
- Cornstarch: Binder, moisture retention.
- Sweet Cream Butter: Lipid source, calorically dense. 80% Fat.
- Experimental test ingredients: puffed millet, high fructose corn syrup (HFCS), corn syrup, de-lactose whey permeate, Fibersol-2, shortening, water, flavors.

Formulation Parameters

The formulation of the RTE bar was specifically tailored to allow easy altering of ingredients and/or processing methods. The first priority for the different trials was to create a RTE bar that the stated specifications (Table 3-1). This involved the development of a novel protein source Milk Protein Precipitate (MPP), removal of HFCS from the formulation, baking and freeze drying processing parameters, and the inclusion of probiotics. The formulations in Table 3-4 are the final recommended formulations. These represent two different approaches to meeting the nutritional and physical specifications of the RTE bar. Both meet or exceed the nutritional specification, as can be seen in their mock nutrition panels (Figure 3-17).

Preparation of RTE Bar

1. First the ingredients are weighed and staged, ready for later use.

Figure 3-1: Weighed ingredients ready for use.

2. The flavor ingredient is mixed with the salt, sugar, and sucralose until it appears uniform.

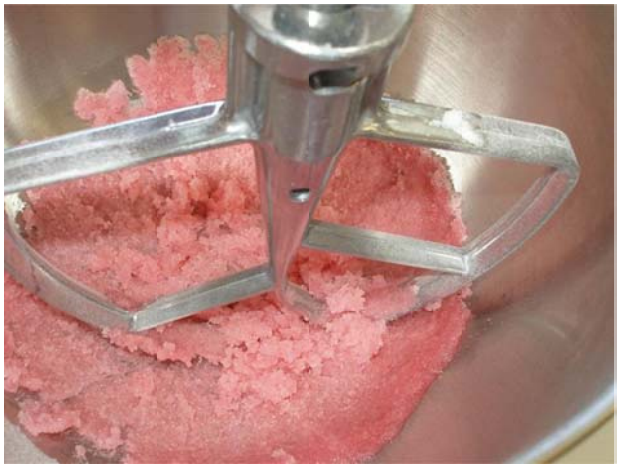


Figure 3-2: Flavor ingredient mixing with sugar, salt, and sucralose

3. The dry ingredients: flour, WPC, cornstarch, are slowly added. Mixing continues until flavor clumps disappear.

Figure 3-3: Dry ingredient addition

4. The “wet” MPP protein is added, which functions as a binder and the major protein source. This mixing step continues for approximately seven minutes or until the dough is formed.

Figure 3-4: MPP protein is mixed into dry ingredients

5. Moisture from the MPP slowly migrates out of the curd structure, causing the mixture to clump. The product slowly becomes a cohesive dough.

Figure 3-5: RTE material after dough is formed

6. The dough is removed from the mixer and placed on a sheet of parchment paper.

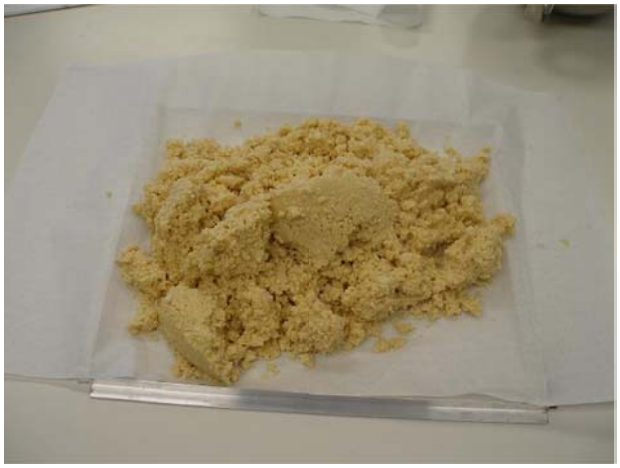


Figure 3-6: Dough removed from the mixing apparatuses

7. The fresh dough is spread around the mold to ensure even distribution, then an additional sheet of parchment paper is placed on top to prevent sticking.

Figure 3-7: Fresh Dough being rolled using custom mold

8. The dough is rolled/sheeted into the precise measurement 11.5” x 14.5” x 0.5”, using a custom made mold.

Figure 3-8: Dough rolled out, before baking

9. The formed dough is then placed on a baking tray with parchment paper and baked for 1.16 hours at 250^{o}F according to the baking schedule.

Figure 3-9: Bars after baking and cutting

10. The bars are then cut to specific dimensions to ensure a proper weight and serving size (75g) and 1.5” X 0.75” x 0.5”.

Processing Methods

The specifications of the RTE bar (Table 3-1) are greatly determined by the processing of the bar. The main purpose of processing the bar is to reduce the final

moisture and water activity in the product. Reducing the moisture increases the energy density of the product as moisture content adds weight but not calories. In addition, drying the bar reduces the water activity (aw) of the bar. This limits the growth of spoilage organisms by reducing the water needed for their growth. Processing also affects the palatability of the RTE bar, which is highly dependent on the texture and mouthful. Several different processing methods were investigated (Table 3-2) and adapted to meet current and specific project goals.

Table 3-2: Summary of RTE processing methods

Method	Time	Temperature	Pressure	Moisture
Freeze Drying	2-8 hours	Product:30^{o}C Plate:100^{o}C	0.35mmbar	1.5%
Vacuum Oven	4-16 hours	25-55^{o}C	25”Hg	>15%
Forced Air Drying	2-10 hours	150^{o}F	Atmospheric	~28%
Convection Baking	1.16 hours	250^{o}F	Atmospheric	~25%

Freeze Drying

Several heating parameters were investigated to achieve the best product using the freeze drier. While some batch to batch variability still existed, the majority of this was removed when the product was rolled to an even thickness and perforated using a roller docker. The holes created by the roller docker acted as channels for the water vapor to leave the product. Without holes the product would balloon up in some sections, creating hollow cavities which would fracture the product. The maximum temperature setting can be adjusted for both the plate temperature and the product temperature. Plate temperature refers to the heating element supplying the radiant heat. Product temperature refers to the

temperature at the center of the product which is set to shut off the plate heating when the parameter is reached.

Figure 3-10: Freeze drying chamber during processing

Freeze Drying Procedure

1. Dough is rolled flat to uniform thickness (0.5”) (Figure 3-8).
2. Holes made in the product using a roller docker.
3. The bars are cut to desired size (Figure 3-9) and placed in blast freezer (-14^{o}F) overnight.
4. Bars are placed in freeze dryer.

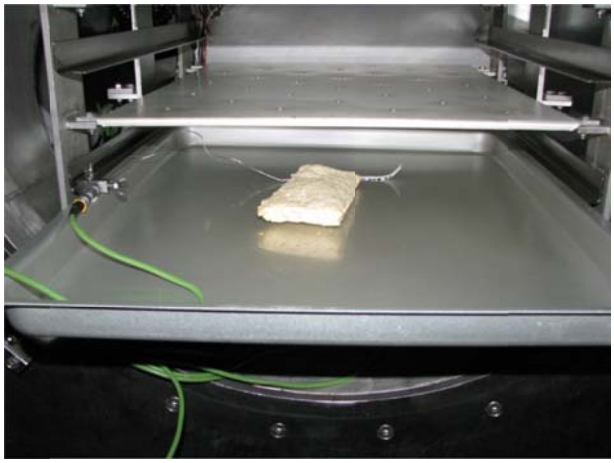


Figure 3-11: Frozen RTE bar placed in freeze drying chamber

5. Settings for optimum freeze drying are programmed via a computer controller as follows: Plate Temp 100^{o}C, Product Temp 30^{o}C, Chamber Pressure 0.35mmbar.

6. Freeze drying process is started and heating will automatically turn off when product reaches constant weight.

Baking

Baking is not a processing method commonly used in the nutrition or high-protein bar manufacturing. Most bar products are not thermally processed but are instead cold extruded for shaping and mixing. However, baking was adopted as a processing method for the RTE bar when the Milk Protein Precipitate (MPP) was added into the formulation. MPP was manufactured in-house and could be considered a "fresh cheese" which carries added food safety risks. In order to ensure a safe product, baking was incorporated to add a thermal heat "kill step" which would reduce potential microorganism load. The objective was to create a product with similar physical and nutritional properties as the established freeze dried product by using an alternative processing method. A general biscotti recipe was adopted which resulted in satisfactory sensory and physical qualities.

Baking took place immediately after the dough was rolled out to the specified dimensions (11.5” x 14.5” x 0.5”) (Figure 3-8).

Baking Procedure

1. Preheat convection oven to 250^{o}F.
2. Bake sheet on middle rack for 25 minutes.
3. Rotate sheet 180^{o}, bake an additional 25 minutes.
4. Remove tray from oven and allow to cool for 15 minutes.
5. Cut sheet into the specified individual unit size (Figure 3-9).
6. Flip individual bars 180^{o} over the top, return to baking tray.
7. Bake for an additional 20 minutes.
8. Remove from oven, allow to completely cool before packaging.

Vacuum Drying

This method was explored as a less expensive alternative to freeze drying, requiring only low thermal heating and no initial freezing. A vacuum oven generally used for moisture analysis was used to investigate this method (Figure 3-13). The required drying time depended on the amount of sample in the chamber and whether the chamber was heated. At ambient temperatures almost fourteen hours were necessary; only four hours were needed at 35^{o}C to dry the product to a final moisture content below 20%.

1. Dough was rolled to the desired thickness (0.5”) (Figure 3-8), and bars cut to desired dimension.

2. The cut bars are placed on the vacuum chamber tray.

Figure 3-12: Bar being placed into vacuum chamber before freeze drying

3. A partial vacuum (25” Hg) was drawn, and chamber temperature increased if supplemental heating was used.

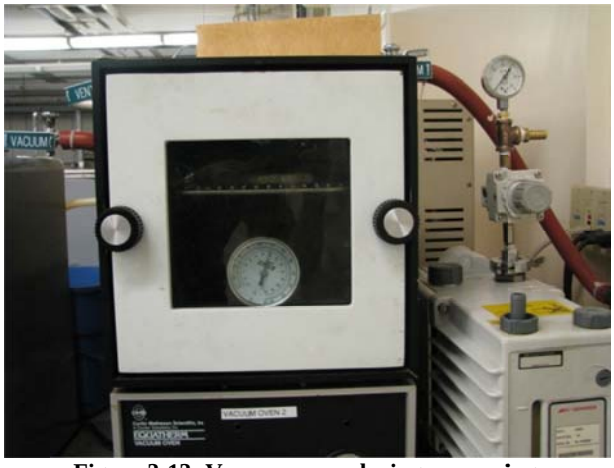


Figure 3-13: Vacuum oven during processing

Forced Air Drying

Dough was dried using a Harvest Saver air dryer designed to dry fresh fruit and vegetables. Using this commercial dryer, air velocity and temperature can be controlled. After several trials this method was deemed to be ineffective. It led to incomplete and inconsistent drying with very long processing times.

Protein Ingredients

Whey Protein Concentrate (WPC)

WPC 80 is the standard dairy-sourced protein used in the industry for bars and processed foods, from breakfast cereals to salad dressings. It has such far-reaching uses due to its superior functionality. It can provide texture, body, and nutrition (high concentration of EAA and BCAA). WPC was the first and primary protein used in the RTE formulation and continued to be used to boost protein content in later formulation trials (See appendix page 111). WPC represented up to 50% of the dry weight in some formulations, providing the bulk structure where carbohydrates are generally used in commercial bars.

Milk Protein Precipitate (MPP)

The shredded MPP curd is treated as a fresh ingredient which must be refrigerated and has a relatively short shelf life. The MPP was easily incorporated into the existing RTE bar formula because it mixed readily and did not clump. The MPP was added during the “wet” ingredient mixing stage (Figure 3-4). Initially, the mixture looked dry and non-cohesive. After mixing the moisture in the MPP begins to hydrate the starch and other ingredients causing the formation of uniform dough. MPP was utilized in several products as a unique protein source (See appendix page 129).

Skim and Butter Milk Powders

SMP and BMP are both relatively inexpensive products compared to WPC since they have a much lower protein concentration (30-38% dry basis) (see appendix page 109-110). These ingredients add protein and nutritional value to a formulation; however, they are not necessarily utilized as such in the industry. These ingredients generally are treated as fillers increasing the total solids of products. In addition, BMP and SMP have higher lactose contents which could make them less desirable in a nutrition bar formulation. Commercial BMP was used initially as an ingredient that could be replaced with a specially manufactured BMP powder with high phospholipid and sphingomyelin contents.

Micronutrients and Flavor Development

Several experimental formulations were tried in order to improve the overall nutritional benefit of the bar. These were executed as proof-of-concept trials to observe if ingredient additions would negatively impact the flavor of the product. The nutritional profile of bars can be easily modified to meet specific market or consumer demands, such as bars with high calcium, high fiber, or low carbohydrate (Hazen 2009).

Delactosed Permeate

Two levels of Delactosed permeate addition were tried, in two different formulations of 2% and 4% dry basis, to the base formulation. The finished product was compared from a sensory perspective and the ash content was also analyzed (see appendix page 106).

Dietary fiber

Dietary fiber in the form of Fibersol-2® was added to the dough mix at various levels in substitution for flour. (See appendix page 107).

Flavor

Considering the importance of the sensory properties of the RTE bar, both sweet and savory flavors were tried. Liquid and powder flavors were added to formulations during dry mixing at the manufacture recommended levels. Cheese, chicken, BB-Q, pasta and mushroom, fruit, and vanilla flavors were tried (Table 7-1).

Probiotics

Probiotics need to be viable in order to provide their benefit, thus the initial survival of the probiotics during the processing is of special importance. Two processing methods (vacuum drying and freeze drying) and three potential probiotic lactic acid bacteria (Table 3-3) were investigated to see how they responded to the process and formulation treatments. In addition BMP and SMP were added as treatments to see if any synergistic effects exist between BMP and the probiotics survival.

Table 3-3: Three probiotic stains used in survival study

Strain	Species
MR220	*L. helveticus*
NCFM	*L. acidophilus*
23272	*L. reuteri*

Figure 3-14: Probiotic "slurry" being added to RTE bar formulation

Fourteen hours prior to the experiment the specified probiotic was inoculated from a mother batch into 10ml of MRS + Cysteine (0.05%) broth and placed in a $C0_2$ controlled incubator held at 30^oC. After this period the bacteria was centrifuged, washed and re-suspended in 10ml of water and added to either the BMP or SMP powders, creating a probiotic "slurry". This "slurry" was then added to the other ingredients and thoroughly mixed (Figure 3-14). A portion of the raw dough was removed and plated to establish the pre-treatment count. The remaining dough was split; half was placed in the vacuum oven and dried, the other half was placed in the blast freezer and freeze-dried the following day. After the bar was processed through either treatment, it was sealed in a high barrier pouch and plated the following day to obtain post-processing counts.

Direct fermentation of BMP powder to produce a "butter milk yogurt" with high viable cell counts was also tried. The theory was that survival of the probiotics could be improved in an environment conditioned by the bacteria instead of being incorporated from an isolated pellet. Initially commercial BMP was used to allow for future substitution. A 20% solution of BMP in DI water was prepared and allowed to hydrate

overnight. The following day the mixture was heated to 105^{o}F, Danisco YO-MIX 533 40 375 DCU yogurt culture was added at 0.0002% w/w, and it was left to ferment for approximately three hours.

Results

Formulation

Table 3-4 shows the recommended formulation for further development. The initial formulation should be considered a “dry mix” formula; this could be produced using conventional current processing methods and ingredients. This formulation uses WPC as a sole protein source. The final formulation uses the MPP ingredient as well as WPC as the protein sources.

Table 3-4: Initial formulation was utilized in several processing trials and the probiotic survival study The final formulation was used in the physiological validation of RTE study,

Ingredient	Initial Formula	Final Formula
MPP	-	62.1
WPC	24	18.8
Sugar	-	10.3
HFCS	21	-
BMP	14	-
Butter	15	-
Water	12	-
Flour	8	3.7
Corn Starch	-	1.88
Puffed Millet*	6	-
Salt	-	1.5
Flavor: Gold Coast #342991	-	1.4
Sucralose	-	0.1
Total	100	100

***: Ingredient substitute for whey protein crisps, Table 3-5**

Table 3-5 Displays protein content and flavor observations of RTE bars formulated using whey protein crisps. Flavor observations were made by three subjects in an informal product evaluation.

Table 3-5: Qualitative and quantitative results of substituting millet with whey protein crisps.

Tested Ingredient* Table 3-4 "Initial Formula"	Final Protein Content	Final Product Observations
TF1: Millet	22.62	Millet holds dough together, good clean flavor, no change to texture
TF1: 50% protein crisps	37.60	Crisps had weak structure, fragmented in mixer, absorbed water and became mushy
TF1: 70% protein crisps	32.74	Bitter astringent flavor, required greater amount, very hard to chew

Table 3-6 Displays the effects of Delactosed permeate added to formulations. The data was used to predict the effect and possible use level of Delactosed permeate. There were no perceived sensory differences between the control and added permeate products, although TF2.2 appeared to have a sweeter flavor.

Table 3-6: Protein and Ash content of two experimental formulas and trials to increase ash content using a "Delactosed" permeate (Delact) product.

Sample Number	Protein	Ash
TF2.1: Control	34.8	1.54
TF2.1: 2% Delact	34.1	2.2
TF2.2: Control	35.7	2.0
TF2.2: 4% Delact	34.6	3.3

Table 3-7 Displays water activity (aw) of several sample RTE bars. Number 1 is an early formulation before the addition of salt. Number 2 is the final formulation used in the Physiological Validation Study and contains salt. Number 3 and 4 are from the same batch but were located in different regions during baking.

Table 3-7: Water activity (mean of two samples), of select RTE bars after baking, comparing formulation, processing and position during baking.

Sample	aw	Moisture Content
Bake formulation (no salt)	0.905	22.7
Bake formulation (with salt)	0.890	20.7
Baked Edge	0.876	21.8
Baked Middle	0.885	22.8

Processing

Table 3-8 Displays the average moisture content of the RTE bar after processing. The target moisture content of the bar is >25%. Each processing method is capable of reducing the moisture content of the product, baking however results in a final moisture content which is closer to this specification.

Table 3-8: Moisture content and aw of RTE bars before processing (raw) and after processing. Each measurement is an average of several (n≥2). Moisture content is calculated on a wet basis, using a moisture oven. Water activity (aw) is measured using an aqua lab water activity meter.

Processing Comparison		
Processes	Moisture Content	aw
Raw	39.0	0.99
Baked	24.0	0.89
Baked + Vac	17.0	0.85
Vacuumed Dried	16.0	0.24
Freeze Dried	8.8	0.1

Table 3-9 displays the variability that exists in three batches of RTE bars made consecutively using formulation TF2.3. The results indicate the relative consistency in the formulation and processing of the bar.

Table 3-9: The moisture and protein contents on wet basis of in-between 3 batches produced in sequence. Moisture calculated using vacuum oven method; protein calculated using Rapid-N-Cube.

Batch	Protein	Moisture
A	36.1	19.25
B	35.2	20.5
C	36.5	18.84

Probiotic

Table 3-10 shows the statistical results from a probiotic survival study; the source heading lists the different factors that were tested, Prob> F lists the probability that the predicted result would occur randomly without being influenced by the before-mentioned factors. If a factor has a p value below 0.05 then it can be stated to have a statistically significant effect.

Table 3-10: Statistical results from a probiotic survival study. Process: freeze dried, vacuum dried. Formulation: SMP, BMP. Probiotic: MR220, NCFM, 23272.

Source	F Ratio	p Value
Formulation	0.0042	0.9496
Probiotic	3.0292	0.0853
Process	0.0447	0.8335
Probiotic*Formulation	2.8586	0.0957
Process*Formulation	0.0239	0.8778
Process*Probiotic	1.6512	0.2031
Probiotic*Formulation*Process	0.4231	0.6575

Table 3-11 shows how the Least Sq mean of a particular treatment combination is the mean of viability for that treatment combination. Superscript values show the treatment combination groups (NCFM | BMP with 23272 | SMP and NCFM | SMP); if any share a superscript then they are not significantly different from each other. If they have different superscripts then they are significantly different.

Table 3-11: Tukeys multiple comparisons= T-Test α=0.05, Least Sq Mean= Mean of % viability

Treatment Combo	Least Sq Mean
NCFM-BMP[a]	24.32
23272-SMP[a,b]	14.78
NCFM-SMP[a,b]	11.00
MR220-SMP[b]	8.14
23272-BMP[b]	6.39
MR220-BMP[b]	3.96

Figure 3-16 shows the concentration of probiotics in the raw dough (initial) and after processing (vacuum and freeze dried). Measurements are in CFU/gram and were found using the standard plate count method.

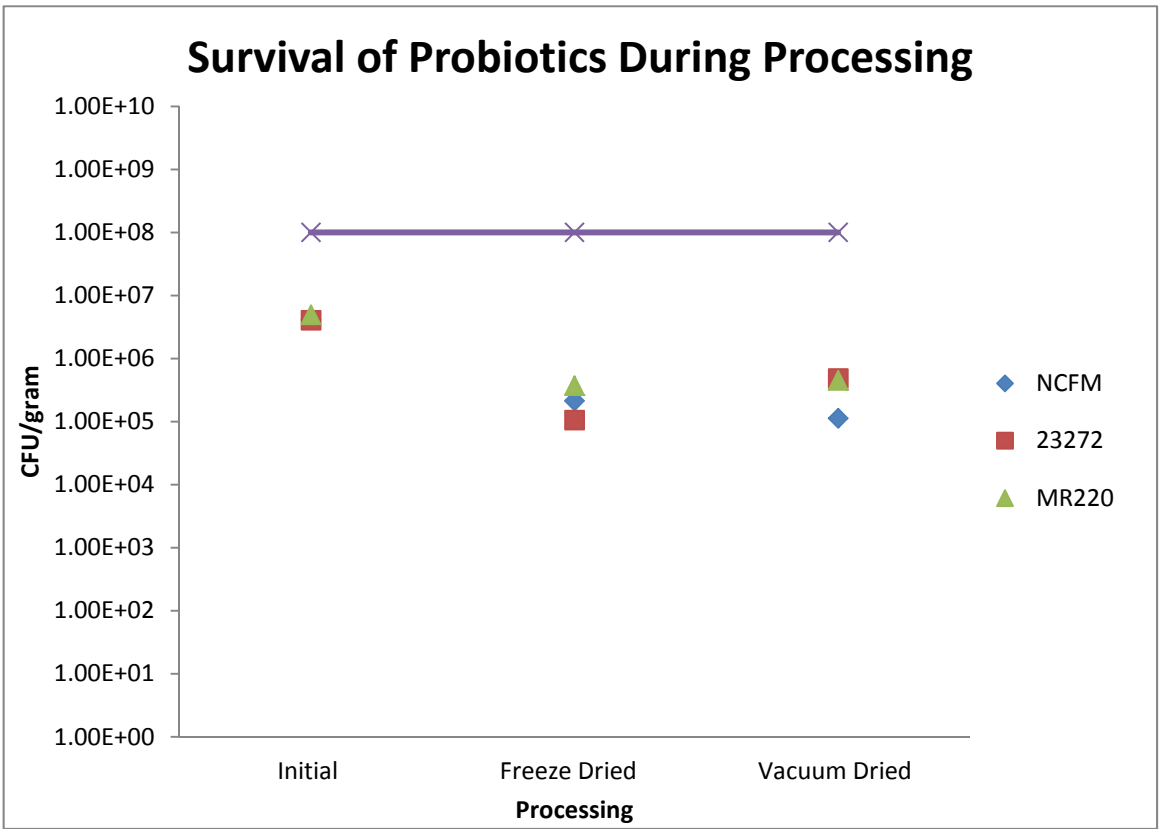


Figure 3-15: Mean CFU/gram (n=18) of selected probiotic strains before (initial) and after processing (freeze and vacuum dried.)

Flavor

Flavoring of the RTE was modifed continually throughout the development process. No formal sensory analysis was conducted but comments and observations were collected form advisors and peers. Flavors were ordered from several flavor houses (Firmenich, Gold Coast, IFF),and both liquid and powdered flavors were used. Savory (Chicken, Cheese) and sweet flavors (Cranberry, Vanilla) were tried. The Physiological Validation Study utilized a Cranberry flavor to create a sweet bar; no final flavor profile was selected. See page 113 in the appendix for a full profile of flavor codes and observations.

Discussion

The development of the RTE bar focused on two separate but related parameters. First the RTE formulation which would influence both the nutritional profile of the bar and dough consistency. Second the processing of the bar, which affected the shelf stability and protein content of the bar.

Formulation

The product development process resulted in two final formulas (Table 3-4), and both met the stated RTE parameters (Table 3-1). The two formulations provide the desired nutritional. The ingredients, mixing order, and resulting dough can be easily scaled-up for mechanized industrial production. The initial formulation utilizes dry shelf-stable ingredients that are combined with HFCS and water. This formulation could be produced using existing ingredients and processing equipment. The final formulation uses a wet MPP protein which was developed in house for this project and is outlined in Chapter 4. This formulation delivers the optimum protein because it combines a complete precipitation of milk protein with supplemental WPC. The bar containing the MPP ingredient was used in the Physiological Validation study (Chapter 5).

The dough in the initial formulation had the consistency of a viscous paste which did not hold shape and was tacky (Table 3-4). In order to improve dough consistency puffed millet was used to “bind” the other ingredients and give the resulting dough structure. The use of puffed millet, which has a high surface area and low density, improves the dough consistency and made it easier to handle. As a result the dough with puffed millet that can be rolled, had structure and is not sticky. However the millet does not contribute to the desired nutritional profile of the RTE bar. A study was designed to

compare the feasibility of substituting the puffed millet for a “whey protein crisps” (Table 3-5). The use of whey crisps is common in the bar manufacturing industry, they available in various sizes and protein contents. Whey protein crisps would contribute to the “energy dense” dairy ingredient profile of the RTE. Three separate RTE batches were produced to compare the protein contents and dough functionality of the formulas with whey protein crisps versus the puffed millets. The resulting protein contents were approximately 22% in control millet RTE, 37% using 50% protein crisps and 39% using 70% protein crisps. This trend is be expected because the protein content of the whey protein crisps contribute to a higher protein content in the finished RTE bar. The whey crisps were much denser and larger quantities were needed to deliver the same dough-binding effect. Additionally both whey protein crisp formulas, had noticeable stale or bland off-flavors that were detected in the finished RTE bar. The final protein levels were considerably higher than the target (25% for a 100g bar) and the whey protein crisps delivered lower functionality than the millet control. The whey protein crisps would shatter in the mixer, absorb water, lose their crispiness, and increased dough density. Due to the low functional performance the whey protein crisps were not used in subsequent formulation as the puffed millet delivered the desired dough profile. The 50% whey protein crisps might have future application in RTE bar formulation if higher total protein or EAA content is desired. The whey protein crisp damage seen in the preliminary trials could likely be reduced by using different mixing methods.

Table 3-6 displays the proof of concept for use of Delactosed permeate in the RTE bar formulation. Delactosed permeate is a dairy derived calcium supplement that was added at varying levels to test its effect on mineral content and flavor profile. When

compared to the control sample, the mineral content (expressed as percent ash) increased in both formulations when Delactosed permeate was added. This indicates increased calcium content in the finished bar which would be favorable in an RTE bar product. Increased calcium intake is beneficial for athletes due to its relationship to muscle contraction and hormone function (Anonymous 1997). Delactosed permeate contains mostly calcium which would interact through ionic binding with water to lower the aw of the RTE. No noticeable off textures were noticed when consuming the RTE containing Delactosed permeate, and the dough consistency was not noticeable effected. However, theses bars were baked and considered sandy and coarse prior to adding Delactosed permeate. The TF2.2 control contained a greater ash content compared to TF2.1; this is due to the higher WPC and BMP content in the TF2.2 formulation both of which have high mineral contents.

The RTE bar water activity was an important parameter. When baking was used as the processing method the water activity of the RTE bar was substantially higher than the target (0.65). To try and control this salt was added at varying levels between 1.5-2% Table 3-7. Adding salt to the formulation lowered the final product aw from 0.950-0.895. This is due to salt's ability to lower the water activity by ionic binding of the otherwise available water. However, adding salt as the sole means to control aw will not be a practical solution as very high concentrations of salt would be necessary to achieve shelf stability. Samples for water activity and moisture content taken from the center and edge of the baking tray indicated that batches were homogeneous in terms of moisture distribution.

Flavor

Flavor, texture, and the nutritional profile of the RTE bar, along with the dairy-heavy ingredients make this bar an ideal product for a savory formulation. The majority of bars currently on the market are sweet. This is due consumer expectations and the common use of sweet syrups and fruit pastes as binding agents. Although chicken and meat flavors may not be appealing, nacho or "Cheez-Its" flavors have a potential to work well.

Delactosed permeate increased the ash content of the formulation which indicates an increase in calcium (Table 3-6). Additionally, Delactosed permeate is said to have salt-reducing and flavor-enhancing properties. These functionalities were not observed in this particular study, however TF2.2 (Table 3-5) was perceived as sweeter in both control and Delactosed samples. This was caused by the higher lactose content of the WPC and BMP formulation compared to the MPP only.

The volunteers in the Physiological Validation Study were asked to evaluate the bar they consumed. This bar was processed through baking, and the formulation is present in (Table 3-4). Subjects stated that the bar was dry and found it difficult to consume a complete serving. Many also described the RTE bar as unsatisfying after the strenuous hikes, preferring the carbohydrate bar. The majority of the subjects liked the control bar, the reasons being the flavor, level of sweetness, and texture. Several subjects indicated that the RTE bar had a "corn bread" like texture.

Shelf Life Expectations

No formal shelf life study was conducted; however, informal observations of bars over time followed the expected trends. Freeze dried bars with moisture <10% and water

activity <0.360 have been stable and without visual defects for approximately one year. Baked bars with higher moisture (<25%) and high water activity levels (<0.900) began to mold after approximately three weeks at ambient temperature (~75^{o}F, 60%RH). All bars were packaged in clear high barrier PE/EVOH/PE vacuum pouches with no vacuum. Samples used in the Physiological Validation Study were packaged in laminated foil pouches. This molding was predictable as the water activity of the final bars was not low enough to ensure shelf stability. Mold requires an aw above 0.7 to grow. The average final aw in a baked bar was 0.89 which was above the target level, but bars were refrigerated (<40^{o}F) and were consumed within one week after production.

Figure 3-16: Baked bars molding after four weeks of ambient storage.

Processing

The purpose of processing the RTE bar was to reduce the moisture content and hence the available water in order to produce a shelf stable product. Several processing methods were utilized to reduce the moisture and aw to the desired levels (Table 3-8). In order to meet these criteria, the bar would need low water activity (<0.65) and/or a suitable “kill step”.

A formulation designed for baking was developed adding a thermal processing step which would limit food safety risks. The target was a finished product with low moisture content (~20%) and a water activity (< 0.65). This would produce results equal to the freeze drying process. However the low water activity level was never achieved. Table 3-8 shows the moisture drop that is achieved from the raw unprocessed dough to the finished baked product. The moisture content was close to the desired 24% compared to <20%. Although the low moisture objective could be reached with a slight alteration to the baking time, the water activity remained too high even after the addition of salt. This resulted in a bar that is not shelf stable and requires refrigeration for extended storage. Although baking was a viable processing method to achieve the nutritional parameters, it resulted in a RTE bar with a short shelf life.

The baking process was optimized (Chapter 3: Baking) by controlling for within-batch and between-batch repeatability. Three batches were made consecutively to study flavor profiles (Table 7-1), comparing different berry flavors at the same use level. The batches used the same formulation and processing parameters and the final moisture content levels of all three were collected (Table 3-9). These results were used to interpret the variability that can occur between different batches of baked RTE bars. The results showed that the random variability was not great (0.5-2.5%), and that the desired low moisture content could be met.

Vacuum drying was investigated as an alternative processing method for the RTE. Vacuum drying does not require any prior freezing of the product as in freeze drying. It can remove the majority of the available water resulting in a moisture content of approximately 16% and aw below 0.24. This stability is achieved because the processes

take place at a lower pressure environment where the boiling point of water is reduced, which allows the free and available water to evaporate at a lower temperature. Additionally the vacuum creates a draw which removes the moisture from the chamber. The vacuum oven used was not large enough to make the quantity of bars necessary, thus halting additional vacuum processing research.

Probiotics

To test the practicality of inoculating viable probiotics into the RTE bar, their survival through the initial processing had to be verified. This study was a multi factorial (2x3x2) experiment comparing the effects of: Formulation (BMP-SMP), Probiotic strain (NCFM, MR220, 23272), and Process (Freeze Dried, Vacuumed Dried). Initial statistical analysis of the data collected indicated there were no significant results in the analysis (Table 3-10). This can be interpreted as no combination of treatments resulted in any different level of survival than another. The Probiotic | Formulation interaction which was compared in the initial analysis showed a p value approaching significance, 0.09 at an alpha of 0.05.

If a multiple comparisons model is made of this interaction NCFM | BMP is seen as separate from three of the five combinations which are all in one group (Table 3-11). While this is not statistically significant it does indicate a trend which could be further investigated. NCFM is a widely studied strain because of its binding potential to the milk fat globule membrane (MFGM). In this study it resulted in a significantly higher viability when BMP was used compared to SMP. Eventhough the main effects of the formulation did not indicate any significant difference (p-value 0.9496), the relationship between

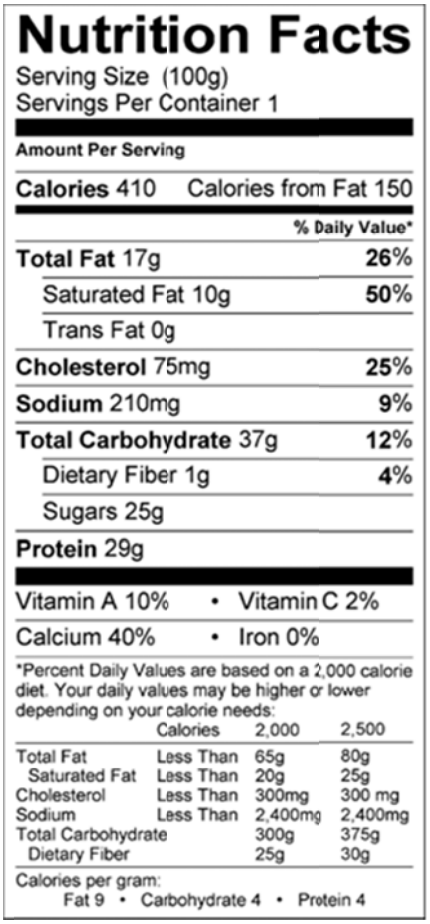
BMP as a functional ingredient for the protection of probiotics has potential for further investigation (Table 3-10).

Comparing the initial counts in the raw dough to those of the processed RTE indicated another interesting trend. Figure 3-15 shows the CFU counts of the raw and processed product averaged over formulation and trials for the different probiotics and processes. The initial inculcation is consistent at approximately $1x10^7$, while the processed bars appear to suffer a one log reduction resulting in post processing counts at $1x10^6$. Both of these values are below the level recommended by the National Yogurt Board (NYB) at $1x10^7$ (Federal Register 2009). The initial inoculums were limited in cell density, and this was then diluted into the mass of the dough formulation, resulting in the low counts. A larger or more concentrated inculcation could lead to a higher cell count and sufficient post-processing survival. Most interesting is the relative subtlety of the vacuum drying process, despite occurring at elevated temperatures and being a longer process. There were no significant differences between the two processing treatments (p-value 0.83) (Table 3-10). This can also be seen in the survival graph (Figure 3-15) with the same count resulting from both methods with all probiotics strains. This would seem to indicate that vacuum drying could represent a more cost effective alternative to freeze drying for the preservation of a functional RTE food.

Conclusion

A novel protein bar formulation has been developed utilizing a high quality dairy protein source with the goal of reducing muscle wasting in soldiers and endurance athletes. Providing energy dense nutrition in a convenient form is a valid option to deliver

the required nutrients necessary to minimize the detrimental effects of undernourishment and chronic stress experienced by this demographic. Dairy is considered one of the best sources of high quality protein. Dairy provides a complete protein containing all essential amino acids, which is critical for nutrition additionally it is high in BCAA which are linked to muscle action. The two formulations that were developed deliver the benefits of dairy nutrition in convent energy dense bar (Figure 3-17). The formulation on the left utilized only dry conventional ingredients and provides higher protein content than initially specified. The formulation on the right was reduced to 75 grams for the purpose of the Physiological Validation Study and provided the desired protein content but had reduced calorie content.



Nutrition Facts

Serving Size (100g)
Servings Per Container 1

Amount Per Serving

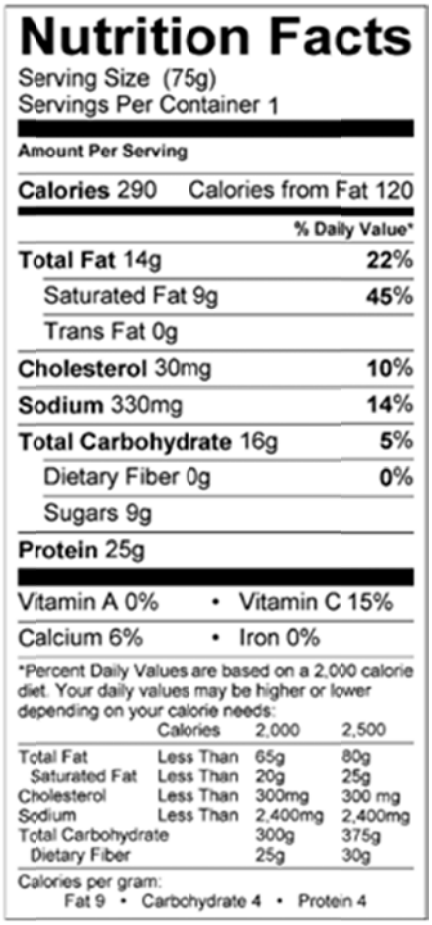
Calories 410	Calories from Fat 150
	% Daily Value*
Total Fat 17g	**26%**
Saturated Fat 10g	**50%**
Trans Fat 0g	
Cholesterol 75mg	**25%**
Sodium 210mg	**9%**
Total Carbohydrate 37g	**12%**
Dietary Fiber 1g	**4%**
Sugars 25g	
Protein 29g	

Vitamin A 10% • Vitamin C 2%
Calcium 40% • Iron 0%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories	2,000	2,500
Total Fat	Less Than	65g	80g
Saturated Fat	Less Than	20g	25g
Cholesterol	Less Than	300mg	300 mg
Sodium	Less Than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4



Nutrition Facts

Serving Size (75g)
Servings Per Container 1

Amount Per Serving

Calories 290	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 9g	**45%**
Trans Fat 0g	
Cholesterol 30mg	**10%**
Sodium 330mg	**14%**
Total Carbohydrate 16g	**5%**
Dietary Fiber 0g	**0%**
Sugars 9g	
Protein 25g	

Vitamin A 0% • Vitamin C 15%
Calcium 6% • Iron 0%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories	2,000	2,500
Total Fat	Less Than	65g	80g
Saturated Fat	Less Than	20g	25g
Cholesterol	Less Than	300mg	300 mg
Sodium	Less Than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure 3-17: Nutritional Facts Panel for final formulations, panel at left using only WPC, right panel using MPP and reduced to 75 gram serving size.

Baking can effectively be used as a processing method for the production of an RTE bar. Baking allows for the production of large batches and increases control over the drying and processing of the RTE. Some of the advantages of baking are that it yields a safe product, it is a standard industrial technology, and is relatively inexpensive. Baking also has disadvantages, such as the development of a cooked flavor resulting from Maillard browning, dry crumbly texture, higher residual moisture and water activity. Baking can also be coupled with an additional processing step like ambient vacuum drying to decrease final moisture. Freeze drying is also a potential option for processing

an RTE bar. It results in a final product with good sensory and physical qualities, with a crisp texture, sweet mild taste, and very low moisture and water activity levels. Freeze drying has high additional costs and is not common in this segment of the industry although it has the potential for large scale manufacturing.

Many flavors were incorporated throughout the development process (Table 7-1), but no profile was finalized. The final formulation resulted in a bar that is mild with a light dairy flavor which is a flexible base for further flavoring. This could allow the same formulation to be flavored several ways, which was one criterion of military rations. Flavoring the bar would require extensive sensory trials; while some preliminary work was done, an extensive sensory study would be very useful.

Probiotics are one of many functional ingredients that could be added to this and other RTE bar formulations. In initial studies we have seen some indication of probiotic survival after processing. However, there are other methods for incorporating probiotics like sporeforming shelf stable probiotics, encapsulation, and in-bar fermentation that could be investigated. Probiotics are of great interest in this type of product mainly because of their positive association with gut health and immunity. Other trace nutrients and functional ingredients could readily be incorporated into the formulation. Adding minerals, vitamins, and high fiber are options that are already on the market and have been shown to function in RTE bar formulation. Finally the development of MPP as a viable protein was necessary to achieve the desired protein profile for RTE formulation. Its utilization led to a formulation that met the nutritional specifications but without the flavor defects seen with WPC.

4. MILK PROTEIN PRECIPITATE (MPP)

Introduction

Ricotta is a classic Italian style cheese traditionally manufactured from whey. Its unique manufacturing process combines high heat and acidification for protein precipitation. The process of ricotta cheese manufacturing was used as a model for the production of Milk Protein Precipitate (MPP). Precipitating all the protein present in fluid milk and using the resulting curd in an RTE bar formulation would deliver high quality protein in a convenient and novel form without the negative flavor of dairy powders. Using concentrated lactic acid, high heat, and longer holding times results in a soft curd (MPP). The mild flavored curd can be used as a nutritional ingredient in further processing - a type of industrial cheese.

The process for the production of MPP was refined in the Dairy Products Technology Center (DPTC) and the resulting curd produced a reliable composition (Table 4-1). This ingredient was incorporated into the RTE bar formulation to replace a portion of the WPC protein. While WPC is the concentration of a small fraction of possible milk proteins, MPP was developed to contain a much larger fraction of milk proteins. This property makes MPP closer to the intrinsic nutritional value of milk. The purpose of creating the MPP was as an experiment to test the possibility of producing a high protein product from milk. This protein ingredient was tested using several analytical testing methods in order to establish its composition. The functionality of the protein ingredient was also tested in the RTE formulation and other products.

Table 4-1: Approximate analysis of MPP (n=9) batches. Whole milk ricotta as described by Kosikowski 1982

Components	MPP Specifications	Whole Milk Ricotta
Protein (N-cube)	25- 27	11.2
Fat (Babcock)	23-25	12.7
Moisture (Microwave Drying)	35-40	72.2
Carbohydrate (Difference)	2-5	3.0
Ash (Muffle Furnace)	0.75-1.5	-

Materials and Methods

The production of Ricotta and Ricotone style cheeses is outlined by Kosikowski 1982. MPP follows these guidelines and was adopted to create a curd with a significantly different nutritional profile (Table 4-1). The fluid milk is heated to a higher temperature before acid addition (180 ^{o}F verses 176^{o}F), the pH is reduced further (4.6 versus 6.0-5.9) (Kosikowski 1982). Additionally no salt was used in the MPP production process. A holding period at the elevated temperature was also included, this allowed for an increase in curd strength.

Ingredients and Equipment

- Whole milk, skim milk, whey depending on desired fat content
- Lactic Acid (88%), diluted to 35% in H2O
- Portable temperature compensating pH meter, 10mL pipette
- 1.5 L plastic container
- Large stirring paddle, and scooping device

- Double jacketed steam kettle
- Cheese molds
- Thermometer
- Cheese shredder.

MPP Manufacturing Process

1. 10 gallons of full fat raw milk is received from the dairy.
2. The milk is added to a double jacketed steam kettle and heated with periodic stirring to 180^{o}F.

Figure 4-1: Raw Milk agitation and heating

3. One liter of milk is removed and acidified with 35% lactic acid until the pH is < 4.6. The quantity of acid needed to acidify the remaining 10 gallons is then calculated.

4. When the milk reaches 180^{o}F, it is agitated vigorously until a “cyclone” forms in the center of the milk. The calculated quantity of acid is then added to the milk and agitation is stopped.

Figure 4-2: Acid being added to heated milk during “cyclone” agitation

5. A lid is placed over the kettle to help retain heat, and the milk is heated until it reaches 190^{o}F.

6. The temperature is held constant at 190^{o}F for 30 minutes.

7. The lid is removed. The curd should have floated to the surface creating a solid layer. This layer is then scooped carefully to avoid shattering of the curd. Too much agitation or dripping whey can break up the remaining product.

Figure 4-3: MPP curd being removed from surface of milk

8. The scooped curd is then transferred to Gouda cheese molds where the free whey is allowed to drain.

Figure 4-4: MPP curd after removal from double jacketed steam kettle, draining whey.

Figure 4-5: Whey remaining in kettle after removal of MPP curd.

9. The MPP curd is pressed under 10lb weights for 2 hours. This removes free residual liquid forming a close knit curd.

Figure 4-6: MPP curd being pressed to remove excess moisture

10. The curd is then vacuum-sealed into cheese bags and refrigerated overnight.

Figure 4-7 Vacuum packed MPP cheese wheels before refrigeration

Figure 4-8: Formed MPP curd before shredding

11. The curd wheel is removed from the package and shredded to form a uniform moist protein ingredient.

Figure 4-9: MPP curd being ground in Kitchen Aid mixer

12. The shredded curd is then packaged and is ready for use. The curd is vulnerable to mold and spoilage bacteria so must be refrigerated. The milk is sterilized during the processing but the moist curd is an ideal target for post processing contamination (Kosikowski 1982).

Results

Table 4-2 compares MPP and Ricotta manufacturing efficiency, by analyzing protein and fat content of the starting ingredient (raw milk, whey) and the resulting residual liquids.

Table 4-2: Comparing fat and protein content of MPP to classic Ricotta using FOSS 4/6/10

Ricotta	Fat	Protein	MPP	Fat	Protein
Whey	0.26	0.9	Milk	4.16	3.5
Residual Liquid	0.022	0.55	Residual Liquid	0.066	0.39

Table 4-3 compares MPP and Ricotta in terms of protein and moisture contents. Both curds were either pressed with a 10lb weight for two hours or left to drain naturally.

Table 4-3: Compositional analysis of acid and heat coagulated curd (MPP and Ricotta) from either Milk or Whey respectively 4/6/10

Ricotta	Moisture	Protein
Non -Pressed	79.86	10.8
Pressed	64.22	12.1
Approximate Yield		1%

MPP	Moisture	Protein
Non – Pressed	60.29	21.8
Pressed	35.2	27.2
Approximate Yield		11.1%

Figure 4-10 is a chart displaying the quantity of acid (35% lactic acid) needed to change the pH of milk. Six different MPP productions are recorded to construct an acidification chart. Approximately 15ml of 35% lactic acid is needed to acidify one liter of raw milk at 50°F.

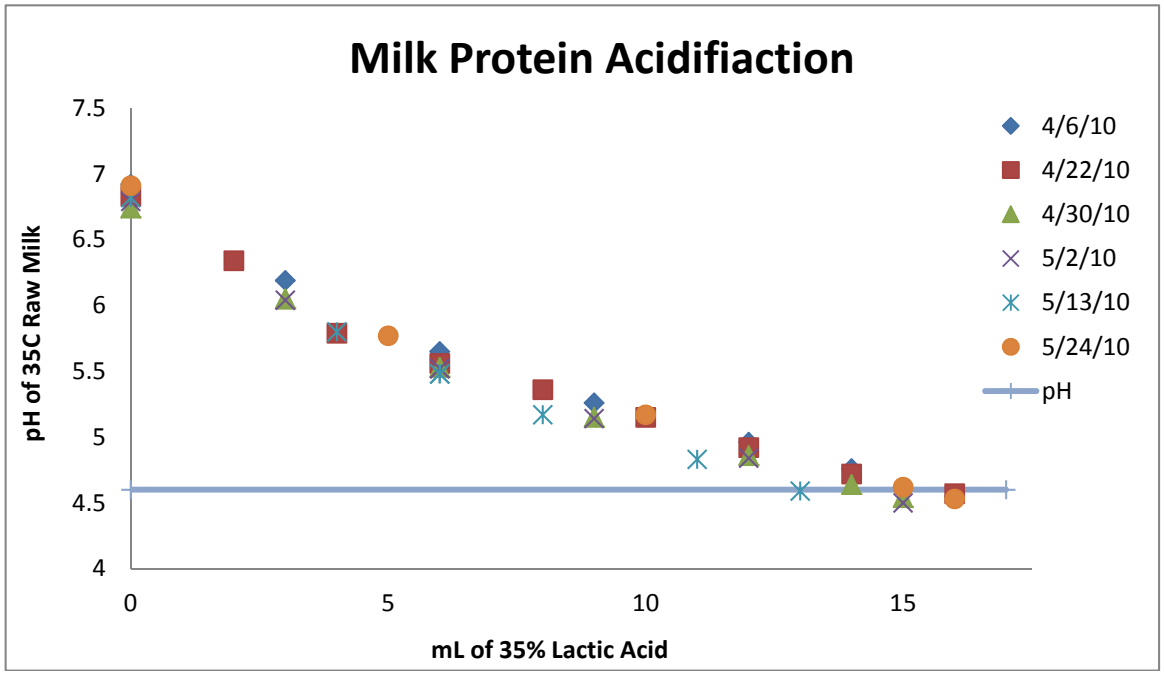


Figure 4-10: Acidification curve of several MPP batches, using 35% lactic acid Raw Milk

Table 4-4 is an approximate analysis of two MPP batches manufactured on different days. Fat analysis was conducted using both the Mojonnier and Babcock

methods. Protein determination was conducted using the Elementar Rapid-N-Cube. Mineral or ash content were determined using a muffle furnace, and moisture content using the vacuum oven method.

Table 4-4: Milk protein precipitate compositional analysis of different batches (values averaged)

Date Tested Sample	Protein: Rabid-N-Cube	Fat: Babcock	Ash: Combustion	%MC: Vacuum oven
4.13.10	23.4	22.87	1.095	43.17
4.22.10	25.81	27.5	1.23	40.27

Table 4-5 shows the results of the protein analysis of different MPP productions; the production method was kept consistent.

Table 4-5: Protein content of different MPP batches, results done in multiples using Rapid-N-Cube

Date Performed	Sample	Percent Protein
4.30.10	MPP	24.76
5.02.10	MPP	24.61
5.13.10	MPP	20.84
5.27.10	MPP	27.44
5.28.10	MPP	26.61

Figure 4-11 is an urea gel depicting the protein profiles of several samples related to MPP production. MPP's starting ingredient milk (well one), the residual liquid (well 2), and the MPP protein from two manufacturing dates (wells 3 and 4). These are in contrast to cheddar cheese and cheddar whey (wells 5 and 6). Well 7 shows the protein profile of the RTE bar compared to that of a competitor (well 8), and WPC (well 9).

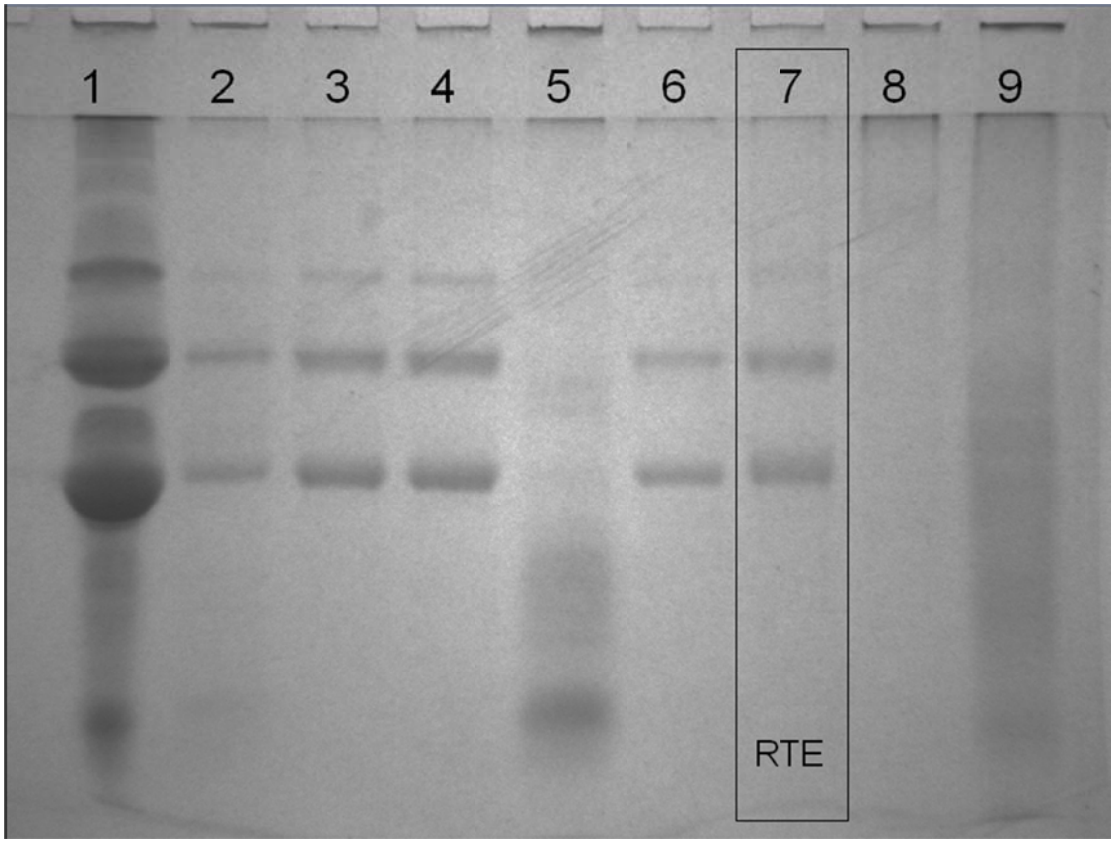


Figure 4-11: Urea Gel, 8uL filled into each well, assorted protein samples. 1:MPP Milk (5/27), 2: Residual liquid from MPP production (5/27), 3:MPP from (5/27), 4:MPP produced (5/5), 5: Whey from Cal Poly Cheddar Cheese (5/27), 6: Cal Poly Cheddar Cheese Curd (5/27), 7: RTE bar made using MPP (5/17), 8: Cliff Bar "Builder Bar", 9: WPC

Discussion

Production of MPP was based on the manufacturing methods for ricotta. The initial process focused on establishing a unique production procedure for MPP which differs from that which is used for Ricotta. In the MPP process, a complete co-precipitation of the protein occurs unlike the partial protein precipitation in Ricotta made from whole milk or whey. This leads to higher concentrations of protein and fat and a lower moisture product (Table 4-1). The difference between ricotta and MPP can initially be identified during the production procedure. Cheese whey was used to manufacture

ricotta; this contains a much lower protein content than milk (0.9% versus 3.4%). After precipitation the final protein content of the two residual liquid streams are similar, with the MPP registering lower (0.4 verses 0.55%) (Table 4-2). The greater efficiency at removing the total protein from the liquid phase is due to interactions that form between the casein and whey protein fractions. The effectiveness of MPP can also be compared to rennet cheese manufacturing. The remaining protein left in MPP residual liquid contains approximately 0.45% protein while rennet whey contains approximately 0.9%. Co-precipitation is achieved by reaching the iso-electric point of casein with the low pH, and the whey protein precipitation using high heat. As a result this process yields a complex protein aggregate of both casein and the whey proteins (α-lactalbumin, β-lactoglobulin) while the single precipitation of whey forms a more simple fragile curd.

This co-precipitation has a large impact on the resulting curd produced, increasing the protein content and reducing the moisture (Table 4-3). The higher protein content and yield that is seen with the MPP is due to higher initial protein in milk (Kosikowski 1982). However the lower moisture that is seen results from the syneresis that occurs in the co-precipitated protein aggregates during the hold time. The residual liquid is expelled as the proteins hydrophobic regions bind, however in ricotta this is not as significant. To further reduce the moisture content and increase the protein concentration the curd was pressed. Pressing was defined as placing the curd in a Gouda cheese hoop under a 10lb weight for two hours, and Non-Pressed as the curd left to drain in a plastic basket for two hours. This additional step reduced the curd moisture 42% in the MPP versus 20% with ricotta. This again results from the co-precipitation where the aggregates have less moisture most

of which is easily removed, while the whey ricotta has lower solids and a greater amount of residual liquid that is incorporated into the curd during precipitation.

The acidification of MPP is an integral step in the co-precipitation of the dairy protein. Acidification is also an important step in heat precipitated cheeses. However, acidification has a more significant role in MPP manufacturing than in ricotta. Acetic and citric acid are commonly used in the manufacturing of ricotta, but result in distinctive flavors in the curd. Lactic acid produced by inoculated starters results in a milder flavored curd and is the recommended acidulate for whole milk ricotta (Kosikowski 1982). Hence, 35% lactic acid solution was used in the MPP manufacturing process which allowed for quick and consistent acidification of the milk (Figure 4-10). While day-to-day variability would exist in the production of MPP, a use level of approximately 14-16ml of 35% lactic acid is necessary to acidify one liter of milk. This value could be used to create a standard manufacturing procedure for MPP.

The composition of MPP is detailed in the approximate analysis of two separate batches manufactured on two dates (Table 4-4). These two batches fit within the range of the MPP specifications outlined in Table 4-1. While the composition of the two batches shows variability, it is apparent that a similar ingredient is produced. Larger variability is possible, as is seen in Table 4-5 where the protein content ranges between 20% and 27% across five separate batches. This large range is due to the handmade batch processes used to make this product. Many factors could influence the protein content of the final product and not all can be controlled without proper mechanization of the process. Poor efficiency during initial curd removal could lead to higher residual moisture; improper acidification and heating would lead to less complete protein precipitation. Many

additional factors would influence this bottom line, however industrial production would greatly limit this variability.

MPP was developed to deliver a high protein curd with a composition identical to that of milk and dairy proteins. The MPP would then be utilized as an ingredient to deliver this protein to the consumer in the form of a RTE bar. A urea electrophoresis gel was prepared using selected protein fractions (Figure 4-11). This gel illustrates the potential for the RTE bar to have the same physiological effects as dairy when MPP is used. The urea gel is able to visually demonstrate the change in protein through processing. Well 1 is the protein profile of milk, which demonstrates the complexity of the RTE protein target. Wells 3 and 4 both contain MPP protein and show similar profiles when compared to the original milk sample. Additionally, looking at the profile of the resulting whey can give an indication of what proteins were not precipitated. Looking at well 2 which contains the whey left over after MPP processing, the profile looks similar to the MPP itself. This is a striking difference from the profile of cheddar cheese whey in well 5 and curd in well 6. This is because the processing of the MPP is designed to precipitate more total protein from the milk, thus resulting in a curd with a more "complete" profile. The protein profile of WPC in well 9 shows no distinct bands or pattern. This is due to the processing methods of WPC which subject the proteins to thermal and mechanical degradation. The "complete" protein profile is preserved through the processing of the RTE bar (well 7) with a protein profile almost identical to MPP used in its manufacturing.

Conclusion

MPP delivers the positive nutritive value of milk protein, and represents an alternative method of incorporating dairy in products beyond powders and fluid milk. This ingredient is manufactured using high heat (190^{o}F) and low pH (4.6), which results in a complete precipitation of both whey and casein proteins. MPP exhibits excellent functional properties and provides added nutrition in the RTE bar and other products. The MPP can aid in the structure and binding of formulations in a similar way to HFCS or wheat gluten. MPP can additionally be used to simplify product formulations since it incorporates fat, moisture and protein. MPP can also serve as a binder and/or bulking ingredient in a bar or yogurt, while having the added benefit of being derived from dairy. In order to ensure that its functionality and potential is realized, MPP would require further work, particularly concerning shelf life and storage. As a fresh, un-aged cheese with high moisture MPP has little hope of a commercially viable shelf life. Post processing contamination is the major factor that compromises the stability of this ingredient. The addition of preservatives or the utilization of aseptic packaging or freezing could extend the shelf life but further development is necessary. Overall, MPP has the added benefit of being derived from dairy and being perceived as a natural ingredient.

5. PHYSIOLOGICAL VALIDATION OF RTE STUDY

Introduction

The benefit of supplemental protein for sports recovery is a current and highly debated topic. Proponents point to the anabolic effect of dietary protein and the improved energy-balance gained from consuming protein post exercise. Opponents hold that the total caloric energy and the metabolic ease that carbohydrates offer are more important for recovery (Jentjens et al. 2001). It has been suggested that active individuals require greater amounts of protein than the RDA (Anonymous 1997). These excess amounts are at 50-100% compared to their sedentary counterparts (Lemon 1987). The development of the high protein RTE bar was based on the former premise, aiming to deliver protein in a calorically dense dietary supplement. The hypothesis for this study is that providing 25 grams of dairy-derived protein post exercise would improve body composition measured by weight and body fat and reduce inflammation and physiological stress markers measured in the blood. The purpose of the blood markers was to compare the physiological effect of the treatment bars on post exercise recovery.

Blood Markers

The following five blood components were selected as markers for this study for their correlation with inflammation and metabolism:

1) Erythropoietin (EPO): A hormone involved in the production of red blood cells. It is also responsible for promoting neuronal survival after hypoxia and other trauma (Sirén et al. 2001). EPO is involved in the biological signaling of the brain and nervous system and has also been associated with cellular proliferation.

2) Hydrocortisone (Cortisol AM): Cortisol levels can serve as an indicator of hypothalamus pituitary adrenal axis (HPA) activity, which is involved in neurological stress responses. Fluctuations in individual cortisol levels are also associated with perceived stress (Wust et al. 2000).

3) C-Reactive Protein (CRP): A protein whose concentration in the blood is directly related to the immune system response to tissue injury, infection, and a key inflammation marker. CRP level's are routinely tested when evaluating human diseases and are associated with the immune system (Thompson et al. 1999). CRP is synthesized by the liver in response to factors released by fat cells adipocytes (Pepys and Hirschfield 2003).

4) Creatine Phosphokinase (CPK): An enzyme responsible for the reversible conversion of ATP to ADP. CPK is said to function as an energy transporter, delivering the energy from the site of production to that of utilization. CPK is also said to have a buffering capacity, functioning like an energy storage mechanism (Wallimann and Hemmer 1994).

5) Adolase: An enzyme involved with fructose metabolism which can be correlated to the dietary intake of carbohydrates (Munnich et al. 1985).

In this study, paid subjects performed strenuous hikes on three consecutive days, which were repeated after a one week rest period. The subjects hiked specified routes carrying 20% of their body weight in backpacks. The physical activity prescribed was designed to mimic military combat situations where physical and mental stresses are high. The participants were split into two teams which competed for speed and tactical points. A single blind cross-over design was implemented with subjects receiving one

treatment bar after completing each hike during the first test period and the other bar through the course of the second test period.

Subjects and Methods

Participants

Recruitment was open to all Cal Poly students but focused on the regional ROTC, wrestling, cross country, and swim teams. The subjects were all young and athletic between 18 and 30 years of age. Subjects were screened for milk allergies and other medical conditions and asked to fill out Physical Activity Readiness Questionnaire (PAR-Q) forms and Subject Information Forms for legal compliance. The study was approved by the Cal Poly Human Subjects Board and the participants were informed of the potential risks involved in the study. The subjects were told not to control their diet or exercise routines for the purposes of the study but were asked to fill out diet logs for the dates involved. On the night before each test period (5/20/10 and 6/03/10), the subjects were invited to a free carbo-loading dinner. This also served as an orientation session where the researchers were available to answer questions and provide information on the study.

Protocol

The study consisted of two three-day test periods, separated by a one week rest or wash-out period. The two test periods consisted of three consecutive days of strenuous hikes, chosen for their length (between 6 and 8 miles) and difficulty (topographical images are available in the appendix page 114). Each complete test period was considered a treatment, with one team being administered the test bar and the other team

the control bar. On the first day of the test period (5/21/10, 6/04/10), subjects went to the University Health Center to have baseline blood draws and have their weight and percent body fat determined. The subjects' body weight was recorded and used to calculate their "carrying load" which was to be used during the test period hikes. Each subject was required to carry 20% of their body weight which included their backpack, super soaker, and water bottle. The remaining carrying weight was reached using sand bags. Each subject was provided a 32oz sports bottle filled with lemon and lime Gatorade during each hike.

On the mornings of the test periods, subjects met at 11 am in the Kinesiology department building. The subjects joined their respective teams and were given their water filled super soaker and sports bottle. When the subjects were ready, one team which alternated started first and was followed 15 minutes later by the second team. A "medic" was assigned to follow behind the last subject and was responsible for picking up garbage, carrying a cell phone, attending to minor injuries, and carrying additional water. As the subjects completed the hikes, they were given their corresponding treatment bar and water was made available. The subjects were asked to completely consume the bars. After finishing their respective bars, the subjects completed an anaerobic power test, a "30 second Wingate", and their post hike choice reaction test. They were then were free to go. This process was completed on each of the three days during the test period.

Response Variables

Body Composition: Measured by static and underwater weighing Friday (5/21, 6/04) and Sunday (5/23, 6/06). Response reported as change between Sunday and Friday measurements.

Simple Choice Reaction: Time to respond (lift corresponding finger) to a light stimulus, measure before and after each hike. Response reported as daily change between after and pre hike reaction time.

Blood Draws: Samples taken on Friday (5/21. 6/04) and Monday (5/24, 6/07). Response reported as change between Monday and Friday blood draw.

Peak Power: Measured as highest mechanical power (Watts = Force x Distance) generated during the first 5 seconds of a 30 second Wingate Test. Measured after each hike, response reported as daily peak power.

Experimental Design

The experiment followed the simple cross-over design (Woods et al. 1989) common in small scale medical studies. The study intended on being single blind, with the teams receiving one of the two treatment bars unknown to them. The initial group of subjects (n=36) contained two females and thirty-four males. On the first day of the study (5/21/10), the group was randomly separated into two teams, either “Green” or “Gold” by the flip of a coin. One female from the Gold team was moved to the Green team to balance the sex ratio between teams. The resulting teams, Gold (n=17) and Green (n=19), were given colored t-shirts. During the first test period (5/21 – 5/23/10) the Gold team was given the test bar and the Green team the control bar, this was decided by a coin toss.

After a one-week rest period, the treatments were flipped, the teams received the alternate bar, and the same hikes were performed.

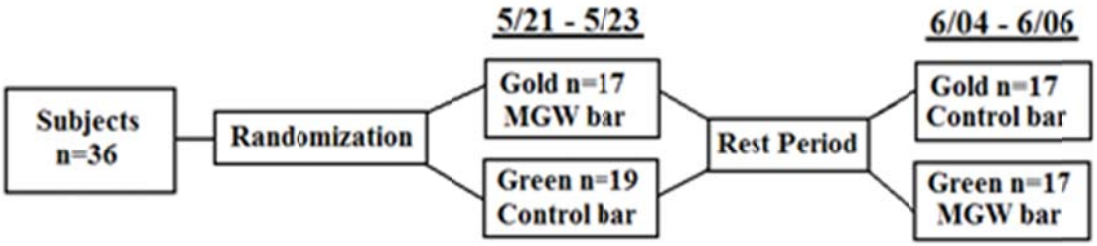


Figure 5-1: visual summary of experimental design

Statistical Analysis

The Mann-Whitney test was used to analysis the data. This is a two-sample non-parametric comparison tool, similar to the conventional t-test. The Mann-Whitney, however, uses the median values from the two groups as opposed to the means used in the t-test. This allows the Mann-Whitney to be used for data which does not meet the assumptions of the t-test, namely normality and equal variance. Non parametric methods are commonly used for human response data where variability between subjects is high and outliers are common (Koch 1972). Early analysis of the collected data shows that the assumptions of normality and equal variance were violated. This resulted from several extreme outliers which occurred randomly during each blood draw (a table of the different outliers for each marker can be found in the appendix(Table 7-2). Due to the apparent variability in the results, all statistical analyses used the nonparametric analysis tool "Mann-Whitney" with a comparable significance level of 5%(α=0.05). Using the cross-over design allowed each subject to function as their own control (Woods et al. 1989), as each subject experienced both treatments. The main concern with a cross-over design is the potential for a "carry-over" effect, indicating the treatment administered

during the first period had lingering effects into the second period. This can be avoided through a long flush out time and by using treatments without lingering effects. The following statistical model was used to test for treatment effects and possible carry-over effects:

Equation 5-1: Two-stage model adapted from (Shen and Lu 2006)

$Y_{ijk} = \mu + b_{ij} + \pi_k + \Phi_m + \lambda_m + \xi_{ijk}$

i=treatment order, j=subject, k= week, m= treatment

μ= overall mean

b_{ij} = effect of j^{th} subject with i^{th} order and is $\sim N(0,\sigma_b^2)$

π_k = effect of the k^{th} week

Φ_m = direct effect of the m^{th} bar treatment

λ_m = lingering effect of the m^{th} bar treatment

ξ_{ijk} = random error and is $\sim N(0,\sigma_b^2)$

Table 5-1: Summary of effects influencing response in weeks, adapted from (Shen and Lu 2006).

Team	Bar Order	Week 1	Week 2	Sum	Difference
Gold	Test-Control	$\mu + \pi_1 + \Phi_1$ ($Y_{1\cdot1}$)	$\mu + \pi_1 + \Phi_2 + \lambda_1$ ($Y_{2\cdot2}$)	$Y_{1\cdot1} + Y_{2\cdot1}$	$Y_{1\cdot1} - Y_{2\cdot1}$
Green	Control-Test	$\mu + \pi_1 + \Phi_2$ ($Y_{1\cdot2}$)	$\mu + \pi_1 + \Phi_1 + \lambda_2$ ($Y_{2\cdot2}$)	$Y_{1\cdot2} + Y_{2\cdot2}$	$Y_{1\cdot2} - Y_{2\cdot2}$

If a carry-over effect is considered significant, the data analysis should not include the second test period as the results would be influenced by the first week. This test was performed by comparing the sums of the two teams' total responses, which only differ by the order of the treatments. This is expressed as the null hypothesis H_0: $\lambda_1 = \lambda_2$, which if rejected indicates a significant carry-over effect.

Equation 5-2: Null hypothesis for carry-over effect, proof and simplification adopted from Shen and Lu 2006).

H_0: $Y_{1.1} + Y_{2.1} = Y_{1.2} + Y_{2.2}$

H_0: $\mu + \pi_1 + \Phi_1 + \mu + \pi_1 + \Phi_2 + \lambda_1 = \mu + \pi_1 + \Phi_2 + \mu + \pi_1 + \Phi_1 + \lambda_2$

H_0: $\lambda_1 = \lambda_2$

The treatment effect is calculated only if the null hypothesis for the carry-over analysis is not rejected. A treatment effect is interpreted as one bar having a significantly different effect on the response variable when compared to the other bar, regardless of the order. This test was performed using the difference between the two teams' total responses multiplied by a constant factor to eliminate all other interfering components. This is expressed as the null hypothesis H_0: $\Phi_1 = \Phi_2$ which if rejected would indicate that the direct effects of the bar treatments were not equal.

Equation 5-3: Null hypothesis for treatment effect, proof and simplification adopted from (Shen and Lu 2006).

H_0: ½(Y1.1 - Y2.1) = ½(Y1.2 - Y2.2)

H_0: ½($\mu + \pi1 + \Phi1 - \mu - \pi1 - \Phi2 - \lambda1$) = ½($\mu + \pi1 + \Phi2 - \mu - \pi1 - \Phi1 - \lambda2$)

H_0: $\Phi1 - ½\,\lambda1 = \Phi2 - ½\,\lambda2$ ($\lambda1 = \lambda2$ if no carry-over exists)

H_0: $\Phi1 = \Phi2$

Treatment Bars

Table 5-2: Nutritional composition comparison between treatment bars

Component	Test Bar "High Protein RTE"	Control Bar "First Strike"
Total Calories	290 kcal	250 kcal
Fat Calories	120 kcal	50 kcal
Protein	25 g	3.0 g
Carbohydrate	16 g	47 g
Dietary Fiber	-	2 g
Fat	14 g	6 g
Saturated Fat	9 g	1 g
Polyunsaturated Fat	-	3 g
Monounsaturated Fat	-	1 g
Cholesterol	30 mg	0 mg
Sodium	330 mg	75 mg
Total Weight	75 g	65 g

The test bar was the RTE Bar formulated and described in Chapter 3. The control bar was the "First Strike Cran-Rasberry", currently supplied in government issue Meals-Ready-to-Eat MRE First Strike rations. The nutrition labels are presented in the appendix (Figure 7-5). The nutrition panels are available in the appendix (Figure 7-4). The First Strike bars were donated by Alexius International, Inc, Fresno Ca. One hundred and twenty First Strike bars were randomly selected from a box containing over five hundred. These bars were removed from their retail wrapping and then placed inside the same laminate bags as the RTE bars and placed in a refrigerator. The Test bar (RTE) was produced in-house following the method described in Chapter 3. The bars were tested for microbial and compositional specifications (Table 7-6). The bars were packaged in

laminated pouches and stored in a refrigerator until used. The RTE bars used in this study contain the MPP protein described in Chapter 4, and the resulting curd was also analyzed for adherence to microbial and compositional specifications available in the appendix (Table 7-4).

Results

Statistical analysis for the carry-over and treatment effects revealed no significant differences; all p-values were greater than 0.05 (Table 5-3). This indicates that no carry-over effect between the test periods exists. In addition, the chosen blood markers were not affected differently by the treatment bars.

Table 5-3: Mann-Whitey p-value results for blood markers

Blood Marker Response	Carry-Over Effect p-value	Treatment Effect p-value
Erythropoietin	0.90	0.21
Hydrocortisone	0.12	0.12
C - Reactive Protein	0.64	0.08
Creatine Phosphokinase	0.06	0.36
Adolase	0.13	0.92

Body composition data for the different teams reveal that they were not well balanced. The mean weight for the Gold team throughout the study (182lbs) was approximately 14lbs greater than that of the Green team (167lbs) (Table 5-4). The Gold team also had a 3% greater body fat. Both team lost body fat and total weight on average every test period, except the Green team in week 1. Variability appears to be greater in

the test period changes of the Gold team; this is indicated by the larger standard deviation (Table 5-4).

Table 5-4: Summary of body composition measurements over the course of the study (wt in Lbs).

Week	Team	Bar	Measurement	Friday Mean	Monday Mean	Mean Change	Standard deviation
1	Gold	Test	Weight	181.8	181.1	-0.7	2.4
			Fat percent	17.6	16.0	-1.5	3.5
1	Green	Control	Weight	166.6	167.6	1.0	1.8
			Fat percent	14.5	12.7	-1.7	2.6
2	Gold	Control	Weight	181.7	180.0	-1.8	3.6
			Fat percent	17.6	16.0	-1.5	3.5
2	Green	Test	Weight	167.6	167.0	-0.6	2.2
			Fat percent	14.5	12.7	-1.7	2.6

Erythropoietin (EPO)

On first examination of the mean EPO concentration (Table 5-5), the values appear to be very similar, even across the test periods. The mean concentration for the Gold team using the test bar was 8mU/mL while the Green team with the control bar was 9mU/mL (Table 5-5). The variability becomes evident in the mean and median changes between the dates. For example, the mean change for the Gold team with the test bar was -0.47, while the Green team with the test bar was 0.65.

Table 5-5: Summary of blood analysis results for EPO - Range 4 - 27 mU/mL.

Week	Team	Bar	Friday Mean	Monday Mean	Mean Change	Median Change	Standard Deviation
1	Gold	Test	8	8	-0.47	0	3.30
	Green	Control	7	8	0.65	1	2.64
2	Gold	Control	8	9	1.76	3	3.46
	Green	Test	8	9	0.65	1	3.66

Besides the p-value for EPO, the mean effects table and graphs can be used to interpret the direction and trend of the results (Table 5-6). In both weeks the test bar had a lower mean change, despite week 2 having a higher overall response. This is also confirmed by the fact that both Green and Gold teams had the same total treatment effect of 1.3, indicating that the order of the treatment had no effect on the resulting blood response.

Table 5-6: EPO summary of effects table, values are the mean difference for the test period.

	Gold	Green	Totals
Week 1	Test: -0.5	Control: 0.6	0.2
Week 2	Control: 1.8	Test: 0.6	2.4
Totals	1.3	1.3	
Treatment Difference (Test - Control):		-2.2	

This trend can be quickly visualized from the main effects plots (Figure 5-2): the test bar having a lower mean and week 2 having a higher overall mean difference.

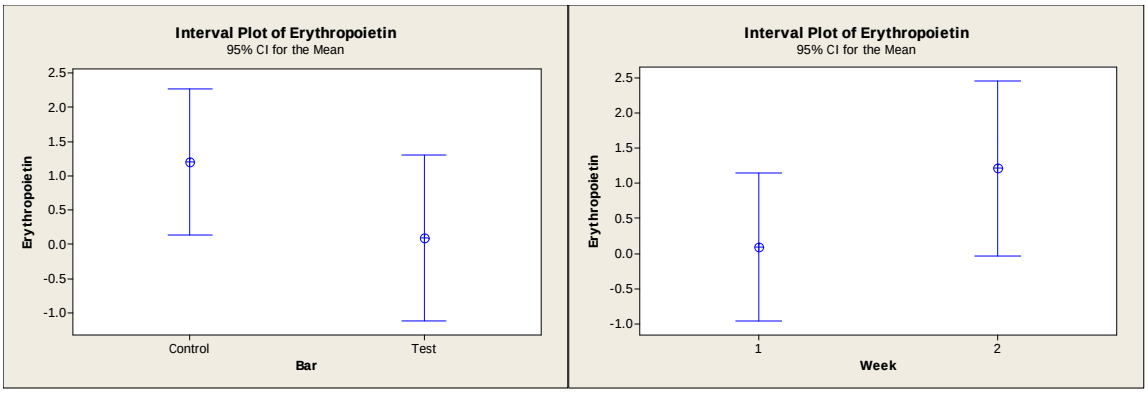


Figure 5-2: Main Effect Chart of Bar type and Week on EOP concentration

Cortisol AM

Three out of the four (team-bar) combinations resulted in a decrease between the baseline and treatment blood draws (Table 5-7). Additionally, the increasing combination (Gold-Test) has the highest mean/median change and standard deviation. This one combination contributed to the lack of treatment effect.

Table 5-7: Summary of blood analysis results for Cotisol AM - Range 6.2 - 19.4 ug/dL.

Week	Team	Bar	Friday Mean	Monday Mean	Mean Change	Median Change	Standard Deviation
1	Gold	Test	15.1	18.4	3.26	3.30	7.36
	Green	Control	16.8	16.4	-0.40	-0.90	5.41
2	Gold	Control	18.1	16.9	-1.18	-0.30	5.06
	Green	Test	16.5	16.0	-0.48	-1.60	4.56

Cortisol AM resulted in a nearly significant carry-over effect (Table 5-3) with a p-value of 0.11. The cause of this can be seen from the lack of a pattern in the summary table (Table 5-8): in week 1 the test bar resulted in a larger mean difference, in week 2 the test bar had a lower mean difference. The total response for the Green and Gold teams is significantly different (2.9 for Gold and -1.7 for Green). The total response each week is also significantly different, with week 1 having a far greater response versus week 2

(2.1 and -0.9). These large discrepancies indicate that the order of treatment might have an effect on the response.

Table 5-8: Cortisol AM summary of effects table, values are the mean difference for the test period

	Gold	Green	Totals
Week 1	Test: 3.3	Control: -1.2	2.1
Week2	Control: -0.4	Test: -0.5	-0.9
Totals	2.9	-1.7	
Treatment Difference (Test - Control):		4.4	

A basic trend, while not statistically significant, does exist and can be seen in (Figure 5-3). The test bar results indicate a higher response compared to the control bar; the total response was greater for the first week compared to the second.

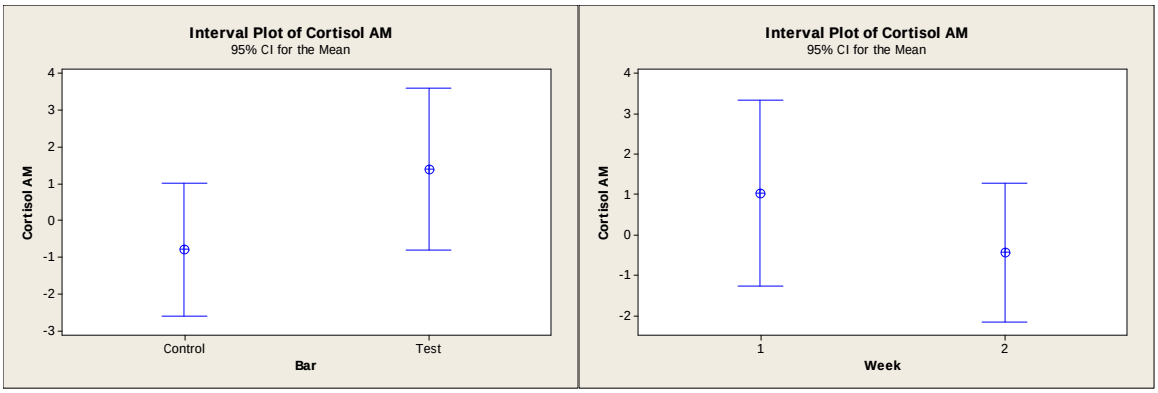


Figure 5-3: Main effect chart of Bar type and Week on Cortisol AM concentration

C - Reactive Protein (CRP)

The individual mean values and mean change show a trend where CRP increases after the treatment period. The mean change and standard deviation for the Green team in week 1 was significantly greater than the other combinations. This spike reduces the ability to detect a trend.

Table 5-9: Summary of blood analysis results for CRP AM - <1.0 low, >3.0 High

Week	Team	Bar	Friday Mean	Monday Mean	Mean Change	Median Change	Standard Deviation
1	Gold	Test	1.9	2.9	1.05	0.90	0.74
	Green	Control	2.6	2.1	-0.48	0.40	6.01
2	Gold	Control	1.2	2.0	0.76	0.40	2.21
	Green	Test	1.0	1.9	0.94	0.60	1.13

The effect of the bars from week and treatment order can be seen by comparing the mean values (Table 5-10). The test bar results show a higher mean difference for both weeks, independent of the order in which they were taken (1.0 verse -0.5) and (0.9 verses 0.8). However the total response for the teams is very different (1.5 verses 0.5), which could indicated an effect of the team.

Table 5-10: CRP summary of effects table, values are the mean difference for the test period

	Gold	Green	Totals
Week 1	Test: 1.0	Control: -0.5	0.6
Week 2	Control: 0.8	Test: 0.9	1.7
Totals	1.8	0.5	
Treatment Difference (Test - Control):		1.7	

The variability within the results is very evident in the main effects plots (Figure 5-4). The test bar resulted in a much smaller spread of data compared to the control, as did week 2. The p-value from the Mann-Whitney test (Table 5-3) indicates CRP as having the closest to a significant treatment effect at 0.076, with the test bar causing a greater response.

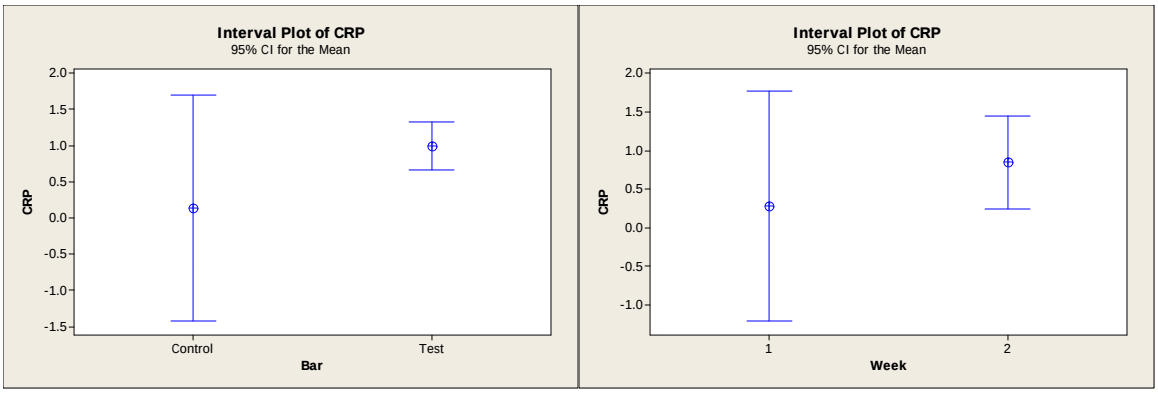


Figure 5-4:Main effect chart of Bar type and Week on CRP concentration

Creatine Kinase (CPK)

The individual mean results display the source of the variability seen in the CPK results. The "Monday" mean for the Gold team in week 1 is 1129 U/L which is significantly greater than the other mean values (260-614 U/L) (Table 5-11). The resulting mean change and standard deviation for that combination are also much greater than the trend set by the other dates.

Table 5-11: Summary of blood analysis results for CPK - Range 35 - 104, U/L

Week	Team	Bar	Friday Mean	Monday Mean	Mean Change	Median Change	Standard Deviation
1	Gold	Test	270	1129	858.47	423.00	1030.18
	Green	Control	222	614	391.35	287.00	326.43
2	Gold	Control	208	374	165.94	112.00	208.23
	Green	Test	274	260	-14.12	33.00	330.48

CPK results indicated the most significant carry-over effect, with a p-value of 0.063. The source of this can be seen by comparing the mean differences with the test bar results having a higher mean difference in the first week (858.5 U/L versus 391.4 U/L) and a lower difference in the second week (-14.1 U/L versus 165.9 U/L). The totals for

the teams are also very different, which could be due to the effect of the treatment order or team. In addition, "week" appears to have a significant effect on the results where week 1 resulted in a higher mean difference than week 2.

Table 5-12: CPK summary of effects table, values are the mean difference for the test period

	Gold	Green	Totals
Week 1	Test: 858.5	Control: 391.4	1249.8
Week 2	Control: 165.9	Test: -14.1	151.8
Totals	1024.4	377.2	
Treatment Difference (Test - Control): 287.1			

The resulting large variability and lack of general direction can be seen in the main effects plots (Figure 5-5). The test bar appears to have a higher response; this is shadowed by the large variability in the test sample. Week 2 has less variability and a lower response.

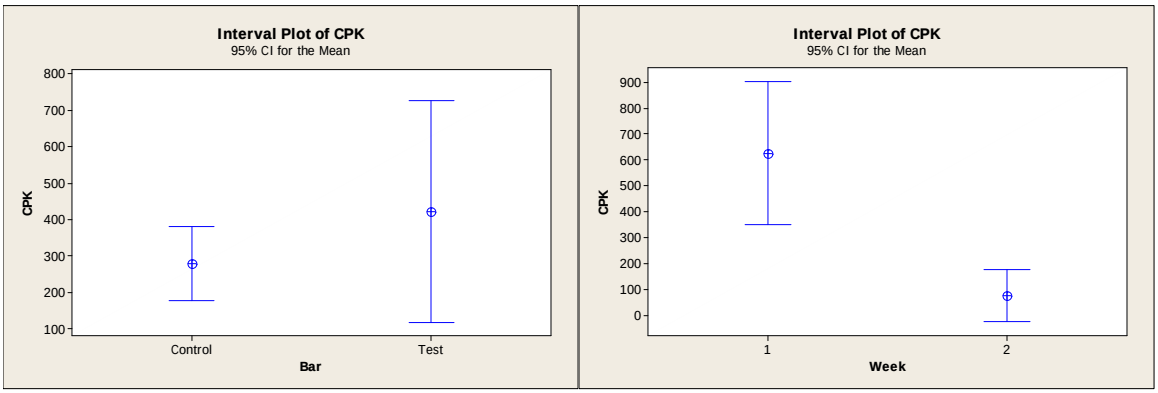


Figure 5-5: Main effect chart of Bar type and Week on CPK concentration

Aldolase

The individual mean responses appear to be separated by week, with week 1 resulting in greater changes than week 2 (Table 5-13). This is true for both treatments and both teams; the variability measured by the standard deviation appears relatively consistent between combinations.

Table 5-13: Summary of blood analysis results for Adolase - range 1.5 - 8.1, U/L

Week	Team	Bar	Friday Mean	Monday Mean	Mean Change	Median Change	Standard Deviation
1	Gold	Test	6.4	11.3	4.91	2.60	4.47
	Green	Control	6.8	10.2	3.39	3.20	2.77
2	Gold	Control	4.9	5.6	0.72	1.10	1.89
	Green	Test	6.6	5.6	-0.94	0.50	4.44

Aldolase resulted in the least significant treatment with a p-value of 0.91 and a close to significant carry-over effect of p-value 0.13 (Figure 5-3). The lack of a treatment effect can be seen in the similarity between the mean differences; the test and control bars had the same response in week 2 and very similar values in week 1. The test bar resulted in a slightly higher response in week 1 and the same response in week 2, which might indicate an order effect.

Table 5-14: Aldolase summary of effects table, values are the mean difference for the test period

	Gold	Green	Totals
Week 1	Test: 4.9	Control: 3.4	8.3
Week 2	Control: 5.6	Test: 5.6	11.3
Totals	10.5	9.0	
Treatment Difference (Test - Control): 1.5			

The variability within the test bar treatments' results is much greater than that of the control bar. However, their mean values appear quite similar indicating no treatment effect (Figure 5-6). The weeks have a similar level of variability with week 1 having a larger mean response than week 2.

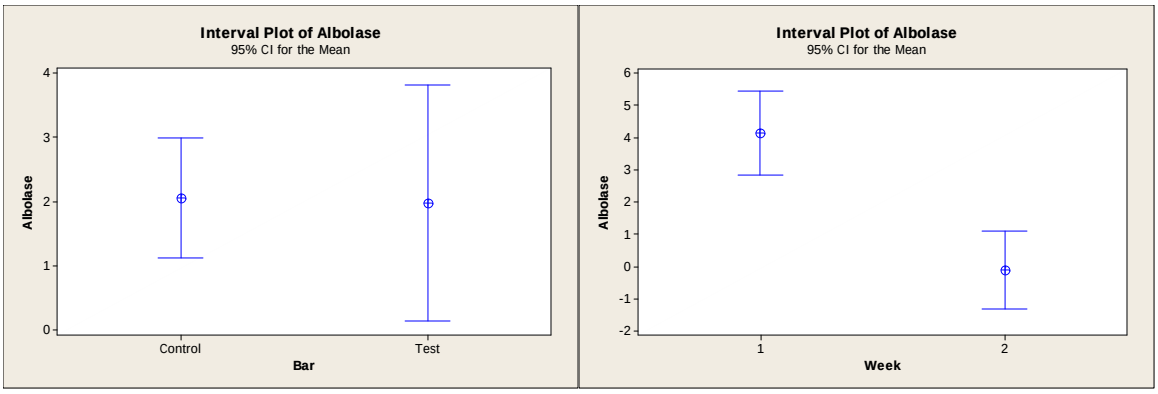


Figure 5-6: Main effect chart of Bar type and Week on Aldolase concentration

Discussion

The data indicated that generally an increase in the particular blood component occurs over the course of test period (Table 7-3). An increase over the test period indicates inflammation has occurred as a result of the physical treatment. An increase in blood marker concentration occurs a majority of the cases, in some instances there is a decrease in one or both weeks. This factor contributes to the variability in the statistical analysis, and is likely caused by confounding factors.

Outliers were considered a potential problem in the initial analysis of the data. Conventional statistical analysis using either GLM or t-test models could not be applied because of lack of normality in several of the blood marker responses. One proposed solution was the removal of potential outliers which would cause the data to fit the normality assumption. A small survey of the data revealed that this would not be practical.

Table 7-2 in the appendix summarizes outliers defined as values greater or less than two standard deviations from the mean. The number of individual considered as outliers from this comparison was significant, the outliers were not consistent across

markers or blood draws. The outliers and the naturally large variability in the data led to the use of the non-parametric analysis tool Mann-Whitney.

As mentioned earlier, the possibility of a carry-over effect is one of the major complications with a cross-over experimental design (Woods et al. 1989). The possibility of this type of influence is significant in drug and therapeutic exercises where the treatments have lingering effects. An estimated washout period of 5x the half-life of the treatment has been recommended for crossover experiments (Shen and Lu 2006). The treatments used in this study are macronutrients (protein, carbohydrates) which are normally consumed by the individuals. Considering the regular digestion time for most individuals, the one-week rest period should have exceeded these recommendations. While there appears to be some indication of a carry-over effect in some markers, it is more likely that the "team" and "week" had an influence on the response. In every blood marker the "week" greatly affected the magnitude of the response. While this was not a treatment, and all factors were purposely kept the same, the response appeared to be influenced. Other factors could exist namely weather differences, motivational and learning changes, and external stressors. These factors might have had an influence over the markers in an unpredictable way.

The results from the Erythropoietin (EPO) analysis did not indicate a carry-over effect (Table 5-3) with a p-value 0.904. The role of EPO in the body as an indicator of red blood cell production could signal muscle anabolism as well as physical trauma (Sirén et al. 2001). The trend in the main effects plots (Figure 5-2) indicated that EPO levels increased more with the control bar than the test bar. This could be interpreted as the result of the control bar increasing blood production in response to the physical

damage of the test period. It could also indicate that the test bar provided a physiological buffer in repairing damaged muscle, which reduced the "exaggerated" response of the control bar.

Subjects were allowed to schedule their after treatment (Monday) blood draw at their convenience during the open hours of the University Health Center (8am-4pm). This factor could have influenced the response of some blood markers. Cortisol levels are greatly affected by time, with increases seen most dramatically 30 minutes after waking up (Wust et al. 2000). This natural fluctuation in the concentration could lead to treatment effects being ignored. Requiring all subjects to return at the same time for each blood draw may have avoided some of the in-subject variation. Cortisol AM was close to causing a carry-over effect with a p-value of (0.12) (Table 5-3). This is likely due to cortisol time dependence instead of an actual lingering effect. The main effects trend indicated that the test bar results had higher levels of cortisol, which is an indicator of stress. However, there was no statistical support for this trend.

C - reactive protein (CRP) had the lowest p-value of any tested marker (0.076) and no indication of a carry-over effect (0.64) (Table 5-3). CRP levels appeared to be higher when the subjects used the test bar versus the control bar. CRP levels increase after the test period in all instances except "Green Team - week 2", (Table 7-3). CRP is related to inflammation and the body's response to physical damage (Thompson et al. 1999). This increase is justified by the inflammation that would have occurred as part of the physical activity during the test period. The main effect plot of the CRP (Figure 5-4) shows the previously stated trend, but in addition, much greater variability in the control

bars' results. This variability was caused by one individual outlier (Table 7-2) and only in "week 1", which might have been caused by an acute and unreported illness or injury.

Creatine phosphokinase (CPK) is the only marker which could be said to have a carry-over effect. While the p-value (0.062) was greater than the preset alpha value of 0.05 there is an increase possibility for week 2 values to be influence by week 1. The mean difference results (Table 5-12) indicates a large difference in means between weeks, week 1 resulting in significantly greater response then week 2, (1250 verse 150). In addition, order of the treatment bar appears to have an effect. In week 1 the test bar resulted in a greater response and in week 2 the response was lower than the control. This fluctuation in the results is likely due to the natural variability of this marker. There are also a large number of outliers (Table 7-2) for this marker. This would indicate that CPK is unaffected by the treatment, despite the trend that is seen in the main effects plots (Figure 5-5), but heavily affected by week.

Adolase levels are directly related to diet, underfeeding can reduce levels while carbohydrate-rich diets can quadruple levels (Munnich et al. 1985). Our results indicated no significant difference between the treatment bars with a p-value of 0.99. This could be interpreted as neither group being underfed carbohydrates during this study. This indicates that the reduced carbohydrate content in the test bar did not lead to any deficiency in carbohydrates for either team.

The body compositional data did not yield any meaningful trends that could indicate any weight or body fat losses associated with either treatment bar. This could have been caused by a lack of balance or subject pairing between the teams. Three

subjects who had a weight over 200lbs were randomly placed in the Gold team. The effect of this is evident from the Gold team having a greater body fat percentage (17.6% versus 14.5%) on the first Monday and being on average 14lb heavier throughout the study. Both teams lost more weight during the second week of the study; while the Green team actually gained one pound in weight during the first period. Both teams lost the same amount of body fat in each period, which indicated no effect of week or treatment on body fat level.

Conclusion

This preliminary study showed that none of the selected blood markers showed significant differences among the treatment bars over the course of the study. Individuals did not show any signs of improvements or under-nourishment from either bar. While there are no statistical correlations, some trends are apparent. EPO levels decreased, CRP levels increased and Adolase levels appear unaffected by test bar consumption. The lack of statistical support for these trends is due to the variability in the results, which is caused by: small sample size (n=34, 17 each treatment), short test period, team balancing, and subject controls.

The experimental design could be improved to produce more tangible results. The first priority would be a larger sample size, with a minimal of 30 individuals per treatment group, which would allow for the identification of smaller differences between the treatments. Diet and exercise controls for the subjects could help to reduce the outliers seen in this study. As a method of reducing variability, the diets and exercise of the subjects can be controlled so that all groups receive the same calories and physical

activity outside of the treatment period. A longer experimental period could also help to distinguish the treatment effects. A longer exercise period could lead to more significant exhaustion, inflammation, and muscle catabolism which is what the RTE bar was developed to reduce. Another factor influencing the response is the time of the post-treatment blood draw. This occurred at earliest eighteen hours after the end of the treatment period, this time might have already reduced the inflammation response. The cross-over design and Mann-Whitney test were effective in the analysis of this study. However more subjects would allow for a randomized complete block design and more traditional ANOVA and GLM statistical analysis.

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7. APPENDIX

Chapter 3 – Bar Formulation and Manufacturing

LĒ-PRO® Dairy Product Solids

Product Definition

Dairy Product Solids derived from Delactosed Permeate.

Chemical Analysis	Typical Values	Specification	Methodology
pH (10% solution)	--	5.2 – 5.6	Combination electrode
Moisture (total moisture)	2.5%	3.0% max.	Atmospheric Oven (150° C)
Ash	30.0%	34.0% max.	Residue on Ignition
Sediment	Disc A	Disc B	ADPI

Microbiological Standards

Methodology		Typical Values	Specification
Standard Methods Agar	Total Aerobic Count	< 2,500/g	10,000/g max.
FDA-BAM	Salmonella	Negative	Negative/750g
Red Bile Agar	Coliform	< 10/g	10/g max. Violet
Potato Dextrose Agar	Yeast and Mold	< 10/g	100/g max. Acidified

Particle Size

	Typical Values	Specification
Passing #40 screen	> 98%	98% min.

Nutritional Information*

(Mean/100g)

Calories **	268.00	St
Calories from fat	0.27	Fib
Total Fat (g)	0.03	Pr
Saturated Fat (%)	0.02	Sodi
Trans Fatty Acids (g)	0.00	Calci
Cholesterol (mg)	0.00	I
Total Carbohydrate (g)	59.60	Vitami
		Vitamir

*Nutritional results, although based on limited testing, fall within the expected manufacturing ranges.

**1.40 calories/gram

Ingredient Statement

Dairy Product Solids

Physical Characteristics

Appearance:	Yellowish powder

Packaging and Storage

Product is packaged in a 500 kg (1100-lb) tote with 3 mil liner, or 22.68 kg (50-lb) bags. Product is recommended to be stored at no more than 80° F (27° C) and relative humidity under 75%.

Specification 1: Delactosed Permeate

Product Specification & Labels

Product Specification

Total Dietary Fiber	: 90% minimum, dry-weight basis via AOAC Official Method 2001.03
Appearance	: White free-flowing powder
Taste / Odor	: Non-sweet / odorless
Solution	: Clear
Moisture	: 5% max.
Dextrose Equivalent	: DE 8.0 - 12.0 via the WS method
pH	: 4 - 6 in 10% solution
Ash	: 0.2% max.
Arsenic	: 1 ppm max.
Heavy Metals	: 5 ppm max.
Microbiological	
Standard Plate Count	: 300 /g max.
Yeast and Mold	: 100 /g max.
Salmonella	: Negative / 25 g
Coliform	: Negative /g

Labeling Information

US: Maltodextrin (FDA GRAS) Maltodextrin, Resistant Maltodextrin, Digestion Resistant Maltodextrin, Maltodextrin (Fiber), Maltodextrin (Dietary Fiber), Maltodextrin (Soluble Dietary Fiber), Maltodextrin (Source of Soluble Fiber), Maltodextrin (Digestion Resistant Type), Maltodextrin (Fibersol-2), Maltodextrin (Dietary Fiber, Fibersol-2), etc.

EU: Dextrin/ Maltodextrin
JAPAN: Indigestible Dextrin

Caloric Values

Caloric value for soluble dietary fibers varies depending on the regulation in each country. Scientifically, the caloric value for as-is Fibersol-2 is estimated as 1.0-2.0 kcal/ gram. For more specific information, please contact us.

US: 1.6 kcal/ gram
EU: 2.0 kcal/ gram
Australia & New Zealand: 1.9 kcal/ gram
JAPAN: 1.1 kcal/ gram
Korea: 2.0 kcal/ gram

Affirmation as a FOSHU ingredient

- Intestinal regularity (1992)
- Moderating post-prandinal blood glucose levels (1994)
- Lowering serum cholesterol levels (1998)
- Lowering triglyceride levels (1998)
- Recommended intake amount: 3-10 grams/serving

Specification 2: Fibersol 2

Product data

Rev.No.fd0306

PURAC® FCC 88

PURAC® FCC 88 is the natural L(+) lactic acid, which is produced by fermentation from sugar. It has a mild acid taste and is widely used as an acidulant in the food industry. PURAC®'s primary functions are to preserve and flavour.

PURAC® FCC 88	
Product	L(+) lactic acid
Form	liquid
Grade	edible special
Colour fresh	max. 50 apha
Colour, 6 months, 25°C	max. 50 apha
Odour	agreeable
Stereochemical purity (L-isomer)	min. 95%
Assay	87.5-88.5% w/w
Density at 20°C	1.20-1.22 g/ml
Sulphated ash	max. 0.1%
Heavy metals total	max. 10 ppm
Iron	max. 10 ppm
Arsenic	max. 1 ppm
Calcium	max. 20 ppm
Chloride	max. 10 ppm
Sulphate	max. 20 ppm
Reducing sugars	passes test FCC
Molecular formula	$CH_3CHOHCOOH$
Molecular weight	90
Chemical name	2-hydroxypropionic acid
CAS number	79-33-4 (general 50-21-5)
EEC Additive number	E 270
USA	GRAS
Complies with	FCC, JSFA and EUSFA

For further information:

Http://www.purac.com/

Specification 3: Lactic Acid 88%

Dry Buttermilk (DBM)

Production Definition

Dry Buttermilk is obtained by drying liquid buttermilk that was derived from the churning of butter and pasteurized prior to condensing. DBM has a protein content of **not less than 30.0%**. It may not contain, or be derived from, nonfat dry milk, dry whey or products other than buttermilk, and contains no added preservative, neutralizing agent, or other chemical. DBM for human consumption complies with all provisions of the U.S. Federal Food, Drug, and Cosmetic Act.

Other Characteristics

Scorched Particle Content2..... 7.5 – 15.0 mg
Titratable Acidity2 ..... 0.10 – 0.18%
Solubility Index.....< 1.25 ml – spray process< 15.0 ml – roller process
Color2 .....uniform cream to dark cream
Flavor2 ..... clean and pleasing

Ingredient Statement

"Dry Buttermilk"

Production Applications and Functionality

Bakery products, frozen desserts, prepared dry mixes, beverages, cheese products, frozen foods, dairy products, salad dressings, snack foods

Storage & Shipping

Product should be stored and shipped in a cool, dry environment with temperatures below 80°F and relative humidities below 65%. Stocks should be rotated and utilized within 6 to 9 months.

Packaging

Multiwall kraft bags with polyethylene inner liner or other approved closed container. (i.e. "tote bins," etc.)

Typical Compositional Range[1]

Percentage

Protein2 ..... > 30.0 – 33.0
Lactose.....46.5 – 49.0
Fat2 .....4.5 – 7.0
Ash.....8.3 – 8.8
Moisture2.....3.0 – 4.0

Microbiological Analysis

Standard Plate Count2 .....< 20,000/g
Coliform.....< 10/g
Salmonella ..... negative
Listeria ..... negative
Coagulase-positive
Staphylococci..... negative

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1 On an "as is" basis
2 USDA Grade parameters (7 CFR §58.2654)

-5-

Specification 4: Butter Milk Powder

Nonfat Dry Milk (NDM)

Production Definition

Nonfat Dry Milk is obtained by the removal of water from pasteurized skim milk. It contains not more than 5% moisture (by weight) and not more than 1.5% milkfat (by weight) unless otherwise indicated. NDM for human consumption complies with all provisions of the U.S. Federal Food, Drug, and Cosmetic Act.

Other Characteristics

Scorched Particle Content2..... 7.5 – 15.0 mg
Solubility Index2.....<1.2 ml < 2.0 ml – high-heat
Titratable Acidity2 ..... < 0.15%
Color2 .....white to light cream/natural color
Flavor2 ..... clean and pleasing

Ingredient Statement

"Nonfat Dry Milk" (_____ % milkfat) if the fat content is over 1.5%

Production Applications and Functionality

Fluid milk fortification, frozen desserts, cheese, yogurt, dairy beverages, bakery products, custards, gravies, sauces, frozen foods, packaged dry mixes, processed meats, soups, infant formulas, snack foods, cosmetics Nonfat dry milk is classified for end-product use according to the heat-treatment used in its manufacture.

The classifications are: high-heat, medium-heat and low-heat. *(see page 2)*

Storage & Shipping

Product should be stored and shipped in a cool, dry environment with temperatures below 80° F and relative humidities below 65%. Stocks should be rotated and utilized within 1 to 1 ½ years.

Packaging

Multiwall kraft bags with polyethylene inner liner or other approved closed container. *(i.e. "tote bins," etc)*

Typical Compositional Range[1]

Percentage

Protein.....34.0 – 37.0
Lactose.....49.5 – 52.0
Fat2 .....0.6 – 1.25
Ash.....8.2 – 8.6
Moisture2.....3.0 – 4.0

Microbiological Analysis

Standard Plate Count2 ..... < 10,000/g
Coliform2 ..... < 10/g
Salmonella ..... negative
Listeria ..... negative
Coagulase-positive
Staphylococci..... negative

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[1] On an "as is" basis
[2] USDA Grade parameters (7 CFR §58.2528)

Specification 5: Non Fat Dry Milk

HILMAR INGREDIENTS®

WHEY PROTEIN PRODUCT BULLETIN

Issue Date: 7/1/2009

Hilmar™ 8000 Whey Protein Concentrate

Hilmar™ 8000 is a highly functional 80% whey protein concentrate ideal for a variety of food and nutritional applications. It is derived from fresh, sweet dairy whey processed by a special cross-flow filtration process.

FEATURES / BENEFITS

- Good Emulsification
- Egg Replacement
- Minimal Carbohydrate Levels
- Low Glycemic Index
- High Acid Solubility/Stability
- Bland Flavor Profile
- High Digestibility
- Superior Protein Source
- Excellent Amino Acid Profile
- Low Mineral Content
- GMO Free
- Kosher Ⓤ and Halal Approved
- EU Approved

APPLICATIONS

- Protein Bars
- Nutritional Fortification
- Bakery
- Dairy and Frozen Desserts
- Savory, Soups, Sauces
- Salad Dressings
- Meat and Surimi
- Confectionery
- Medical Nutrition

NUTRITIONAL VALUES

Composition	Typical	Specification
Protein (% dry basis)	82.5	80.0 min
Protein (% as is)	78.5	77.5 min
Lactose (%)	5.0	7.0 max
Fat (%)	5.0	7.0 max
Moisture (%)	4.5	5.5 max
Ash (%)	3.0	4.0 max

Microbiology	Typical	Specification
SPC (cfu/g)	<1,000	10,000 max
Coliforms (MPN/g)	<3	<10 max
E. Coli (MPN/g)	<3	<3 max
Salmonella (25g)	Negative	Negative
Yeast (cfu/g)	<10	50 max
Mold (cfu/g)	<10	50 max

Other Nutritional Information	Typical
Cholesterol (mg/100g)	218
Total Calories (Kcal/100g)	390
Biological Value (BV)	104
PDCAAS	1
Protein Efficiency Ratio (PER)	3.2
Net Protein Utilization (NPU)	92
Protein Digestibility	95

MINERALS	Typical
Sodium (mg/100g)	175
Calcium (mg/100g)	550
Potassium (mg/100g)	530
Phosphorus (mg/100g)	350
Magnesium (mg/100g)	60
Chloride (mg/100g)	125
Iron (mg/100g)	1

Specification 6: Whey Protein Concentrate

MPP Compositional and Microbial Specification Sheet

Analysis	Tolerance- Range	Actual
Moisture	35-40%	
Fat	23-27%	
Protein	20-27%	
Coliform	< 10 CFU/ml	
E-coli	< 10000 CFU/ml	
SPC	< 20000 CFU/ml	

Specification 7: Milk Protein Precipitate (MPP)

Flavor profile Analysis

Table 7-1: Bench-top flavor experiments, both sweet and savory.

Date	Company	Flavor ID	Flavor	Process	Notes
5/4/10	Gold Coast	336755	Cranberry	Baked	Low detection, low sweetness
		336957			Better flavor, berry like.
		332912			Strong flavor, almost plastic like
11/6/09	Kraft	21000139800	Cheese	Freeze Dried	+ pretty cheesy.
		210007087900	10% use level		+ bitterness, cheesiness.
		21004003600			- musty or rancid,
		210000110600			- less cheese,
		21007084200			- pizza-like flavor, Italian spices.
		210006935800			+ cheesy but mild.
9/29/10	Firmenich	057637	Vanilla	Freeze Dried	Low flavor, poor coverage.
		059200 AP0551	Artificial Cream+ Vanilla		Better coverage needs higher vanilla.
9/22/10	Firmenich	057622 TP0551	Chicken	Freeze Dried	Bad, heavy roast, unpleasant.
		588734 SPM			Non descript, high salt.
		557075 SPM			To sweet, no flavor.
9/15/09	Firmenich	868519CB + 885023 TTB0440	Pizza Roast Garlic	Freeze Dried	Good flavor, garlic slightly too high, good cracker-like

Topographical displays of hikes

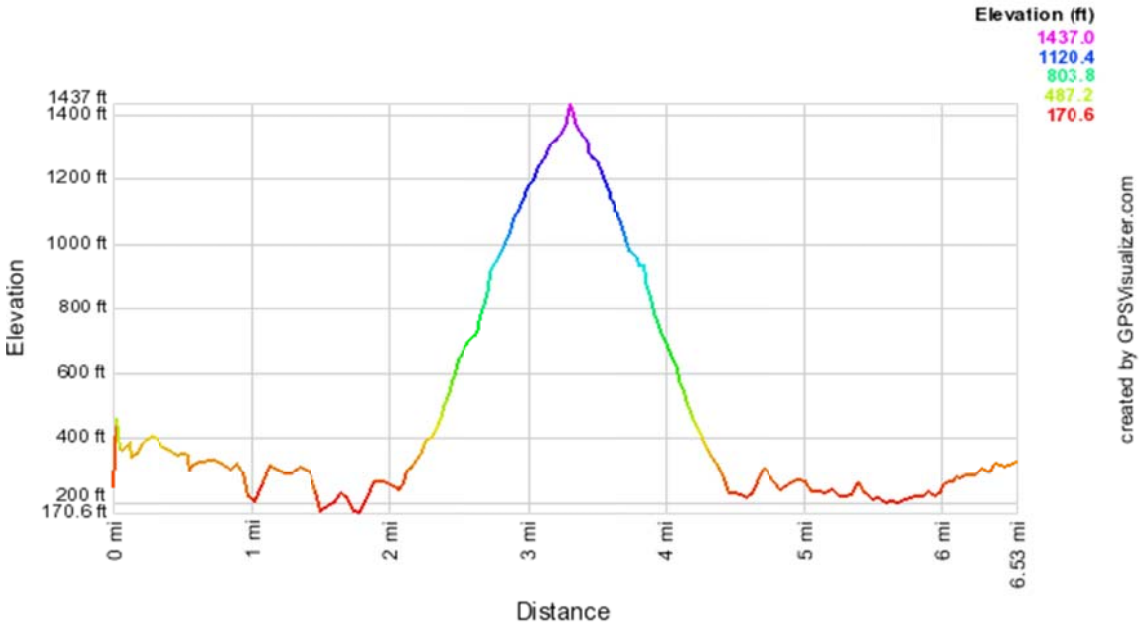


Figure 7-1: Day One hike "Bishop Peak" 5/21 , 6/4

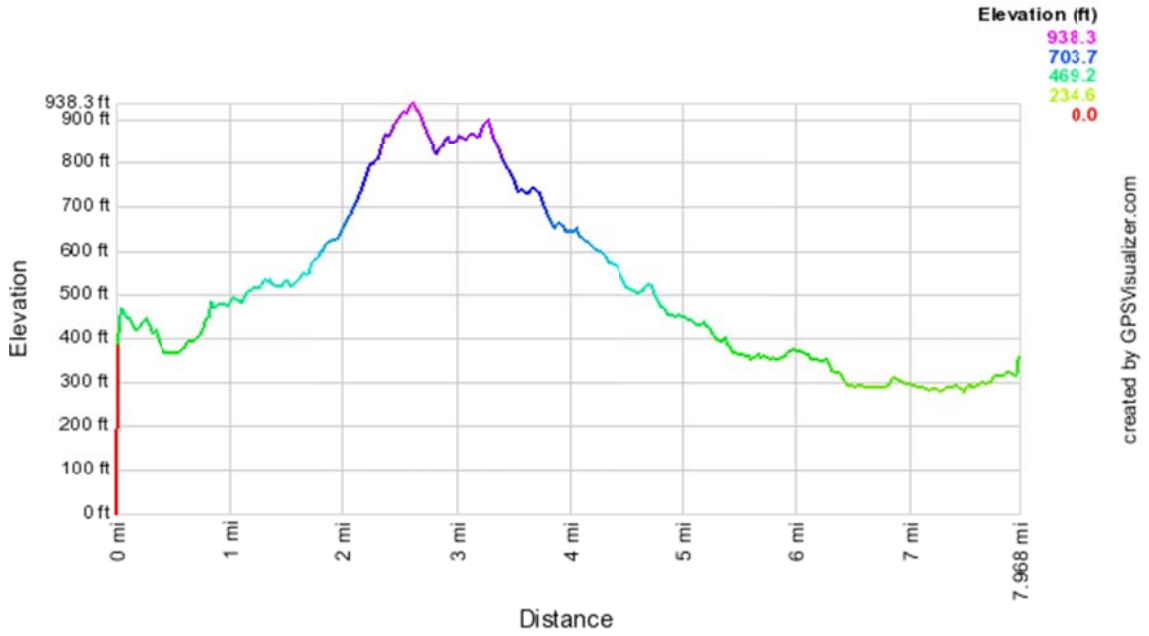


Figure 7-2: Day Two Hike – "Poly Canyon Loop" 5/22, 6/05

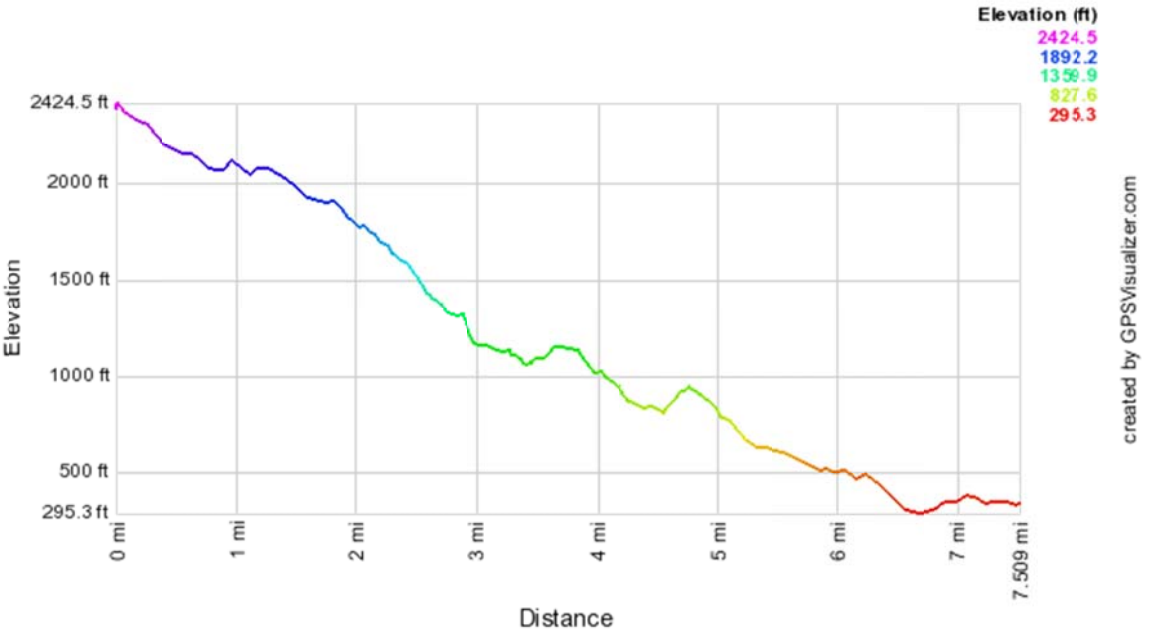
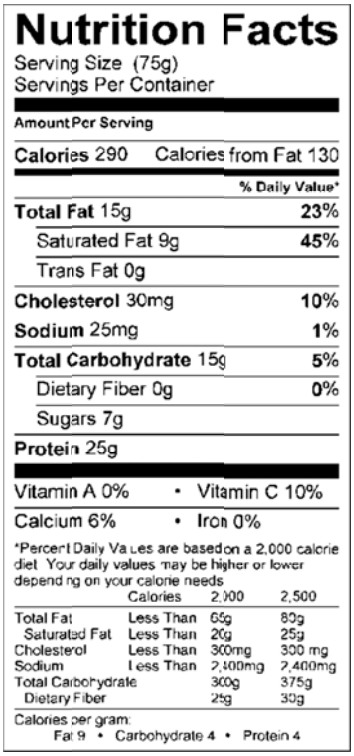


Figure 7-3: Day Three Hike "The Big Down Hill" 5/23, 6/06

Nutrition Panels of Test and Control bars



Nutrition Facts

Serving Size (75g)
Servings Per Container

Amount Per Serving

Calories 290 Calories from Fat 130

	% Daily Value*
Total Fat 15g	**23%**
Saturated Fat 9g	**45%**
Trans Fat 0g	
Cholesterol 30mg	**10%**
Sodium 25mg	**1%**
Total Carbohydrate 15g	**5%**
Dietary Fiber 0g	**0%**
Sugars 7g	
Protein 25g	

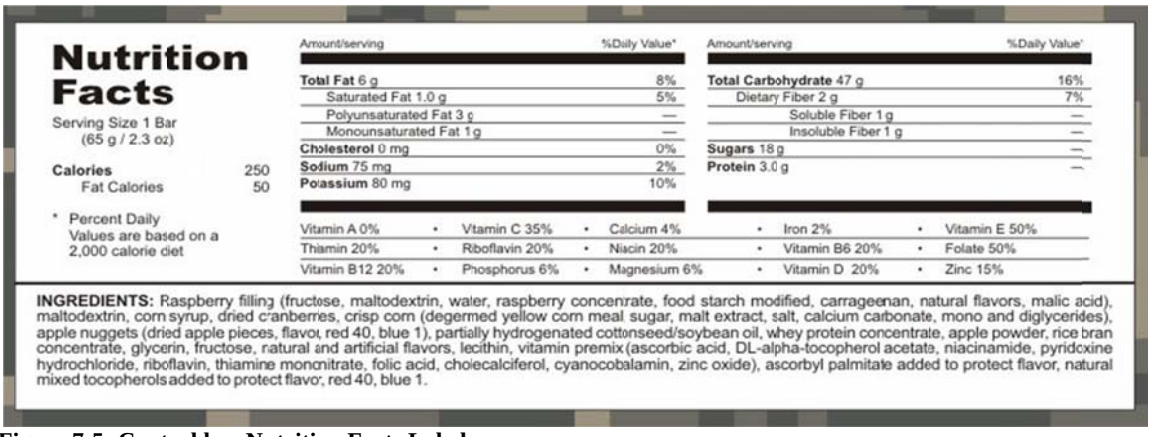
Vitamin A 0% • Vitamin C 10%
Calcium 6% • Iron 0%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs

		Calories	2,000	2,500
Total Fat	Less Than		65g	80g
Saturated Fat	Less Than		20g	25g
Cholesterol	Less Than		300mg	300 mg
Sodium	Less Than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure 7-4: Test Bar 'RTE bar" Nutrition Facts Label



Nutrition Facts

Serving Size 1 Bar (65 g / 2.3 oz)

Calories 250
Fat Calories 50

* Percent Daily Values are based on a 2,000 calorie diet

Amount/serving	%Daily Value*	Amount/serving	%Daily Value*
Total Fat 6 g	8%	**Total Carbohydrate** 47 g	16%
Saturated Fat 1.0 g	5%	Dietary Fiber 2 g	7%
Polyunsaturated Fat 3 g	—	Soluble Fiber 1 g	—
Monounsaturated Fat 1 g	—	Insoluble Fiber 1 g	—
Cholesterol 0 mg	0%	**Sugars** 18 g	—
Sodium 75 mg	2%	**Protein** 3.0 g	—
Potassium 80 mg	10%		

Vitamin A 0% • Vitamin C 35% • Calcium 4% • Iron 2% • Vitamin E 50%
Thiamin 20% • Riboflavin 20% • Niacin 20% • Vitamin B6 20% • Folate 50%
Vitamin B12 20% • Phosphorus 6% • Magnesium 6% • Vitamin D 20% • Zinc 15%

INGREDIENTS: Raspberry filling (fructose, maltodextrin, water, raspberry concentrate, food starch modified, carrageenan, natural flavors, malic acid), maltodextrin, corn syrup, dried cranberries, crisp corn (degermed yellow corn meal sugar, malt extract, salt, calcium carbonate, mono and diglycerides), apple nuggets (dried apple pieces, flavor, red 40, blue 1), partially hydrogenated cottonseed/soybean oil, whey protein concentrate, apple powder, rice bran concentrate, glycerin, fructose, natural and artificial flavors, lecithin, vitamin premix (ascorbic acid, DL-alpha-tocopherol acetate, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, folic acid, cholecalciferol, cyanocobalamin, zinc oxide), ascorbyl palmitate added to protect flavor, natural mixed tocopherols added to protect flavor, red 40, blue 1.

Figure 7-5: Control bar Nutrition Facts Label

Table 7-2: Outliers for each marker occurring by week and team, outlier defined as >/< 2stds.

		Subject ID	
Response	Team	Week 1	Week 2
A – Cort	Gold	37	35
	Green	7	
Adolose	Gold	12, 21	6
	Green	11, 24, 3, 2	3
CPK	Gold	12, 21	12, 25
	Green	2, 3, 20, 24,11	3
CRP	Gold	17	
	Green	8	29, 7
EPO	Gold		28
	Green		4

Table 7-3: Effect of test period on blood marker, separated by team and week.

Marker	Team	Week 1	Week 2
EPO	Gold	No Change	Increase
	Green	Increase	Increase
Cortisol	Gold	Increase	*Decrease*
	Green	*Decrease*	*Decrease*
CRP	Gold	Increase	Increase
	Green	*Decrease*	Increase
CPK	Gold	Increase	Increase
	Green	*Decrease*	Increase
Adolase	Gold	Increase	Increase
	Green	Increase	*Decrease*

Compositional and Microbial results for MPP and Test RTE Bars

Table 7-4: Microbial and Compositional specifications for MPP product

Specification	Tolerance- Range	1a 5/13/10	2a 5/28/10
Moisture	35-40%	***36.9% 36.6%***	***40.2% 43.3%***
Fat	23-27%	***22.9%***	***28%***
Protein	20-27%	***20.95%***	***27%***

Test 1a 5/13/10	Date Read	10^{-1}		10^{-2}	
E- Coli - CC	5/15/10	***0***	***0***	***0***	***0***
Coliform	5/16/10	***1***	***0***	***0***	***0***
SPC	5/16/10	***21***	***24***	***0***	***0***
Yeast/Mold	5/17-5/24	***0***	***0***	***0***	***0***

Test 2a 5/28/10	Date Read	10^{-1}				10^{-2}			
E- Coli - CC	5/29/10 – 5/30/10	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Coliform	5/30/10	***1***	***6***	***1***	***4***	***0***	***0***	***0***	***0***
SPC	5/30/10	***78***		***81***		***2***		***4***	
Yeast/Mold	5/30-6/25	***0***		***0***		***0***		***0***	

Table 7-5: Compositional specifications for RTE bars product

Spec	Tolerance- Range	1a1	1a2	1a3	1a4
Moisture	>24%	***27.22***	***26.22***	***25.66***	***25.66***
aW	>0.890	***0.91***	***0.91***	***0.89***	***0.89***
Protein	<29%	***33.1***	***32.0***	***31.7***	***32.2***

Spec	Tolerance- Range	2a1	2a2	2a3	2a4
Moisture	>24%	***24.4***	***24.0***	***23.3***	***23.8***
aW	>0.890	***0.885***	***0.896***	***0.894***	***0.888***
Protein	<29%	***33***	***38***	***33.6***	***32.6***

Table 7-6: Microbial specifications for RTE bars product

Test	plated	1a1		1a2		1a3		1a4	
E- Coli - CC	10-^{1}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Read 6/2/10	10^{-2}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Coliform	10-^{1}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Read 6/2/10	10^{-2}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
SPC	10-^{1}	***0***	***2***	***2***	***3***	***3***	***0***	***4***	***5***
Read 6/2/10	10^{-2}	***0***	***1***	***0***	***1***	***0***	***0***	***0***	***0***
Yeast/Mold	10-^{1}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Read 6/3-6/25/10									

Test	plated	2a1		2a2		2a3		2a4	
E- Coli - CC	10-^{1}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Read 5/18/10	10^{-2}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Coliform	10-^{1}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Read 5/19/10	10^{-2}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
SPC	10-^{1}	***0***	***3***	***0***	***0***	***1***	***1***	***1***	***0***
Read 5/19/10	10^{-2}	***0***	***0***	***2***	***0***	***0***	***1***	***0***	***1***
Yeast/Mold	10-^{1}	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Read 5/24/10									

Blood Marker and Compositional Data

CDC/AHA recommended cut of points <1.0 low , >3.0 High

ID	5/21/10	5/24/10	Delta	6/4/10	6/7/10	Delta	Sum	Estimate
Gold								
27	0.4	1.8	1.4	2.7	1.2	-1.5	-0.1	1.45
12	0.4	1.8	1.4	0.4	0.8	0.4	1.8	0.5
16	0.6	1.5	0.9	0.6	1	0.4	1.3	0.25
32	0.5	1.4	0.9	0.5	0.7	0.2	1.1	0.35
31	1	2.3	1.3	0.5	1.1	0.6	1.9	0.35
37	16.8	17.7	0.9	2.5	11.5	9	9.9	-4.05
25	2.1	3.2	1.1	0.5	1.2	0.7	1.8	0.2
30	0.4	1	0.6	3.7	4.2	0.5	1.1	0.05
6	0.5	0.9	0.4	1.2	0.8	-0.4	0.00	0.4
36	0.7	1.6	0.9	0.5	0.7	0.2	1.1	0.35
4	1.9	1.5	-0.4	0.8	1.1	0.3	-0.1	-0.35
1	0.5	2.4	1.9	0.5	1.1	0.6	2.5	0.65
17	2.2	5	2.8	2.7	4	1.3	4.1	0.75
38	0.6	1.6	1	2	1.7	-0.3	0.7	0.65
35	0.5	0.8	0.3	0.5	0.5	0	0.3	0.15
21	2.2	4.2	2	0.6	1.3	0.7	2.7	0.65
13	0.9	1.3	0.4	0.5	0.7	0.2	0.6	0.1
Green								
18	0.4	1	0.6	0.4	1.1	0.7	1.3	-0.05
2	0.3	0.6	0.3	0.3	0.9	0.6	0.9	-0.15
34	0.6	0.7	0.1	0.4	1	0.6	0.7	-0.25
7	1.7	0.9	-0.8	3.9	8.2	4.3	3.5	-2.55
23	0.3	4.5	4.2	0.4	0.7	0.3	4.5	1.95
5	0.3	1.4	1.1	0.3	0.7	0.4	1.5	0.35
8	34.8	11.6	-23.2	2.8	3.3	0.5	-22.7	-11.85
3	0.4	0.6	0.2	0.6	2.3	1.7	1.9	-0.75
9	0.4	0.9	0.5	0.5	0.6	0.1	0.6	0.2
20	0.5	0.9	0.4	0.8	1.8	1	1.4	-0.3
28	0.3	0.6	0.3	0.4	2	1.6	1.9	-0.65
29	1.3	5.6	4.3	2.3	4.7	2.4	6.7	0.95
39	0.9	0.9	0	1.8	0.9	-0.9	-0.9	0.45
24	0.9	2	1.1	0.9	1.5	0.6	1.7	0.25
11	0.5	0.9	0.4	0.5	1.6	1.1	1.5	-0.35
19	0.3	1.3	1	0.3	1.1	0.8	1.8	0.1
26	0.4	1.7	1.3	0.5	0.7	0.2	1.5	0.55

ID	5/21/10	5/24/10	Delta	6/4/10	6/7/10	Delta	Sum	Estimate
Gold								
27	15.3	10.6	-4.7	15.7	18.4	2.7	-2	-3.7
12	16.6	10.5	-6.1	18.6	14	-4.6	-10.7	-0.75
16	17.1	12.3	-4.8	10.5	16.1	5.6	0.8	-5.2
32	20.8	24.4	3.6	29.4	24.4	-5	-1.4	4.3
31	17.4	19.5	2.1	11.1	8.7	-2.4	-0.3	2.25
37	9.1	28.2	19.1	14.1	9.5	-4.6	14.5	11.85
25	12.8	7.8	-5	23	19.1	-3.9	-8.9	-0.55
30	9.4	22.4	13	19.2	10.5	-8.7	4.3	10.85
6	11.6	19.4	7.8	9.6	9.3	-0.3	7.5	4.05
36	30.5	39.8	9.3	22.7	23.3	0.6	9.9	4.35
4	18.4	15.7	-2.7	17.9	11.2	-6.7	-9.4	2
1	17.4	24.8	7.4	21.1	23.4	2.3	9.7	2.55
17	8.8	12.1	3.3	9.1	15.8	6.7	10	-1.7
38	19.5	24.8	5.3	20	20.6	0.6	5.9	2.35
35	11.5	14.8	3.3	8.2	20.7	12.5	15.8	-4.6
21	2.6	12.6	10	19.4	21.4	2	12	4
13	18.5	13	-5.5	15.2	11.6	-3.6	-9.1	-0.95
Green								
18	16.9	11.5	-5.4	13.7	9	-4.7	-10.1	-0.35
2	13	15.7	2.7	20.7	18.8	-1.9	0.8	2.3
34	18.9	20.4	1.5	18.2	13.2	-5	-3.5	3.25
7	23	10.7	-12.3	13	14.1	1.1	-11.2	-6.7
23	12.7	17.6	4.9	13.8	10.7	-3.1	1.8	4
5	22.8	16.7	-6.1	10.1	17.9	7.8	1.7	-6.95
8	20.2	19.3	-0.9	23.2	15.2	-8	-8.9	3.55
3	10.5	15.5	5	12.8	17.5	4.7	9.7	0.15
9	20.3	24	3.7	14.4	14.8	0.4	4.1	1.65
20	12	14	2	18.6	14.2	-4.4	-2.4	3.2
28	17.7	23.4	5.7	26.5	21.5	-5	0.7	5.35
29	22.9	18.5	-4.4	19.8	18.2	-1.6	-6	-1.4
39	17.3	18.4	1.1	13.2	13	-0.2	0.9	0.65
24	18.8	14.3	-4.5	14.1	11.3	-2.8	-7.3	-0.85
19	15.6	13.5	-2.1	14.5	19.7	5.2	3.1	-3.65
11	22.3	17.6	-4.7	15.5	21.3	5.8	1.1	-5.25
26	22.7	16.5	-6.2	18.3	21.8	3.5	-2.7	-4.85

Albolase range 1.5 - 8.1, U/L

ID	5/21/10	5/24/10	Delta	6/4/10	6/7/10	Delta	Sum	Estimate
Gold								
27	5.3	7.8	2.5	4.1	5.4	1.3	3.8	0.6
12	8.2	21	12.8	6.2	9.5	3.3	16.1	4.75
16	7.2	8.8	1.6	2.8	4.8	2	3.6	-0.2
32	4	5.6	1.6	3.3	3.8	0.5	2.1	0.55
31	6.1	8.7	2.6	3.1	5.8	2.7	5.3	-0.05
37	6.8	11.3	4.5	4.1	5.2	1.1	5.6	1.7
25	7.6	15.7	8.1	4.8	6	1.2	9.3	3.45
30	5.2	6.7	1.5	3.2	3.5	0.3	1.8	0.6
6	8.6	11	2.4	10.2	5.8	-4.4	-2	3.4
36	4.1	5.1	1	3	2.7	-0.3	0.7	0.65
4	7.8	13.5	5.7	5.2	6.3	1.1	6.8	2.3
1	5.5	13.7	8.2	6.8	7.1	0.3	8.5	3.95
17	6.1	8.2	2.1	3.8	5.8	2	4.1	0.05
38	7.5	8.4	0.9	3.5	5.7	2.2	3.1	-0.65
35	3.1	9.3	6.2	4.6	4	-0.6	5.6	3.4
21	6.8	23.7	16.9	9	6.6	-2.4	14.5	9.65
13	8.1	12.9	4.8	5.6	7.5	1.9	6.7	1.45
Green								
18	5.5	10.1	4.6	4	5.8	1.8	6.4	1.4
2	3.8	9.9	6.1	3.3	5.8	2.5	8.6	1.8
34	11.9	11.3	-0.6	3.8	4	0.2	-0.4	-0.4
7	6.9	7.6	0.7	5.1	5.6	0.5	1.2	0.1
23	4.7	10.1	5.4	4.4	4.2	-0.2	5.2	2.8
5	6.6	8.5	1.9	3.8	5.3	1.5	3.4	0.2
8	7.1	7.5	0.4	11.5	5.4	-6.1	-5.7	3.25
3	11.7	17.6	5.9	27.1	11.4	-15.7	-9.8	10.8
9	9.2	10.7	1.5	10.6	5.8	-4.8	-3.3	3.15
20	4.8	9.5	4.7	3.4	5.1	1.7	6.4	1.5
28	7	6.7	-0.3	4	4.2	0.2	-0.1	-0.25
29	6.8	8.7	1.9	6.4	4.7	-1.7	0.2	1.8
39	5.2	7.7	2.5	4.6	5.2	0.6	3.1	0.95
24	6.1	13.6	7.5	4.6	5.5	0.9	8.4	3.3
19	5.3	8.7	3.4	4.8	6.7	1.9	5.3	0.75
11	6.5	15.4	8.9	5.8	6.5	0.7	9.6	4.1
26	6.2	9.4	3.2	4.6	4.6	0	3.2	1.6

Creatine Kinase (CPK total), Range 35 - 104, U/L

ID	5/21/10	5/24/10	Delta	6/4/10	6/7/10	Delta	Sum	Estimate
Gold								
27	94	571	477	85	205	120	597	178.5
12	518	3815	3297	605	1372	767	4064	1265
16	270	564	294	98	154	56	350	119
32	77	285	208	105	177	72	280	68
31	151	507	356	123	152	29	385	163.5
37	127	895	768	95	207	112	880	328
25	580	1935	1355	275	698	423	1778	466
30	117	279	162	90	141	51	213	55.5
6	694	923	229	686	496	-190	39	209.5
36	127	256	129	106	134	28	157	50.5
4	260	1402	1142	207	385	178	1320	482
1	363	1573	1210	375	672	297	1507	456.5
17	198	351	153	114	190	76	229	38.5
38	283	684	401	91	409	318	719	41.5
35	134	557	423	106	173	67	490	178
21	279	3781	3502	243	472	229	3731	1636.5
13	324	812	488	127	315	188	676	150
Green								
18	121	667	546	118	260	142	688	202
2	125	828	703	122	527	405	1108	149
34	433	412	-21	101	129	28	7	-24.5
7	120	407	287	152	128	-24	263	155.5
23	95	263	168	106	155	49	217	59.5
5	163	219	56	53	153	100	156	-22
8	73	156	83	160	152	-8	75	45.5
3	597	1400	803	1916	749	-1167	-364	985
9	413	501	88	465	161	-304	-216	196
20	201	1090	889	162	352	190	1079	349.5
28	365	449	84	289	213	-76	8	80
29	123	401	278	110	207	97	375	90.5
39	65	181	116	85	118	33	149	41.5
24	258	981	723	144	339	195	918	264
11	176	1099	923	221	224	3	926	460
19	86	391	305	113	203	90	395	107.5
26	367	989	622	346	353	7	629	307.5

Eruthtopoietin Range 4 - 27 mU/mL.

ID	5/21/10	5/24/10	Delta	6/4/10	6/7/10	Delta	Sum	Estimate
Gold								
27	6	6	0	4	6	2	2	-1
12	6	9	3	8	7	-1	2	2
16	6	4	-2	5	5	0	-2	-1
32	9	5	-4	7	4	-3	-7	-0.5
31	13	7	-6	9	9	0	-6	-3
37	14	13	-1	10	14	4	3	-2.5
25	10	17	7	15	18	3	10	2
30	7	7	0	8	10	2	2	-1
6	7	7	0	11	6	-5	-5	2.5
36	10	5	-5	6	9	3	-2	-4
4	13	14	1	8	19	11	12	-5
1	9	4	-5	8	11	3	-2	-4
17	8	11	3	9	8	-1	2	2
38	4	5	1	4	7	3	4	-1
35	6	7	1	6	9	3	4	-1
21	7	6	-1	6	9	3	2	-2
13	7	7	0	7	10	3	3	-1.5
Green								
18	7	11	4	8	15	7	11	-1.5
2	9	7	-2	11	13	2	0	-2
34	5	7	2	6	7	1	3	0.5
7	9	6	-3	5	7	2	-1	-2.5
23	6	8	2	8	6	-2	0	2
5	5	6	1	5	6	1	2	0
8	8	5	-3	4	7	3	0	-3
3	9	11	2	13	10	-3	-1	2.5
9	7	6	-1	6	7	1	0	-1
20	3	8	5	12	9	-3	2	4
28	10	8	-2	13	5	-8	-10	3
29	6	4	-2	6	5	-1	-3	-0.5
39	11	9	-2	15	22	7	5	-4.5
24	3	5	2	5	5	0	2	1
19	5	6	1	4	8	4	5	-1.5
11	10	13	3	9	9	0	3	1.5
26	10	14	4	8	8	0	4	2

ID	Wk1 Wt Change	Wk1 % Fat Change	Wk 2 Wt Change	Wk2 C % Fat Change
Gold				
27	0	0.69	0	0.69
12	-1	-10.15	7	-10.15
16	-4	-3.9	-5	-3.9
32	-2	6.05	-3.5	6.05
31	-2.5	-0.06	-2	-0.06
37	1	0.66	0.5	0.66
25	-1	-2.45	-3	-2.45
30	4	-1.13	1	-1.13
6	-2	-4.75	-8	-4.75
36	1	-6.26	0.5	-6.26
4	-3	-2.32	-1	-2.32
1	-1	-1.15	-3	-1.15
17	3	-0.42	-3	-0.42
38	-3	-0.67	-4	-0.67
35	-1	-0.48	0	-0.48
21	-3	0.5	-8	0.5
13	3	-0.36	1.5	-0.36
Green				
18	-2	0.94	-1	0.94
2	1	0.01	2	0.01
34	1	-2.87	0	-2.87
7	0	-1.36	-3	-1.36
23	2	0.01	1	0.01
5	2	-9.35	3	-9.35
8	0	-1.29	-2	-1.29
3	2	-1.72	1	-1.72
9	-3	-0.78	-3	-0.78
20	0	-4.87	-1	-4.87
28	2	-0.83	-4.5	-0.83
29	0	-1.16	-2	-1.16
39	1	-5.17	0.5	-5.17
24	2	0.2	1	0.2
19	3	-0.8	-4	-0.8
11	5	-0.82	-0.5	-0.82
26	1	0.21	1.5	0.21